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THE COLLECTION AND EXAMINATION OF PLASMA FROM PREGNANT MARES FOR GONADOTROPHIC HORMONE

By

Francis Aylward

The Courtauld Institute of Biochemistry, The Middlesex Hospital, London

AND

C. W. Ottaway

Royal Veterinary College

INTRODUCTION

Cole and Hart (1930a), who first demonstrated a gonadotrophic hormone in the blood of pregnant mares, observed a positive effect beginning between the 37th and 42nd day of pregnancy and reaching a maximum between the 50th and 80th day. Thereafter the hormone concentration gradually decreased, and after the 180th day little or none was present. Kust (1934a) confirmed the presence of this factor in mares of various breeds. Further work (Cole and Saunders, 1935; Cole, 1938) showed that different breeds varied considerably and the individuals of a single breed often varied very greatly; even in the same mare varying concentrations were recorded from year to year. In many mares the serum concentration at about the 100th day of pregnancy was very small; in others a high value persisted beyond this period. Furthermore, the peak values attained in pony mares were four times as high as in mares of the larger breeds. Implantation of the ovum in the mare takes place between the 30th and 60th days, the period of gestation being 330 to 350 days. Cole and Hart (1930b) have suggested that the initial appearance of gonadotrophin in the blood is connected with this phase of embryonic development. Cole, Howell and Hart (1931), and Catchpole and Lyons (1934) examined the relation of serum activity to the foetal crown-rump length and distribution of the hormone in the embryonic tissues.

MATERIAL AND METHODS

The weights and ages of the seven unbroken Welsh ponies (A to G) were estimated and the stages of pregnancy diagnosed by rectal examination (Day, 1940). In five (A, B, C, D, F) the latter figures were checked after killing by estimating the age of the foetus; in one case (G) the date of foaling was known. The ponies were kept on a 7-acre pasture and received hay and a supplement of oats and bran daily. Blood samples were taken weekly from each mare from about the 45th to the 80th day of pregnancy. Each sample amounted usually to 2·25 to 2·50 L, but between the 60th and 75th

days double quantities were usually taken in the hope that this would be the time of peak concentration. Five were bled-out about the 80th day.

Blood was withdrawn from the jugular through a 4 mm. cannula into sterile winchesters each containing 25 ml. of 10 per cent. potassium oxalate. The individual samples which were separately kept and labelled were left overnight in the farm dairy and the plasma, which separated well, was siphoned off next morning into sterile winchesters. Every effort was made to ensure asepis under field conditions. The plasma was frozen as soon as possible, as in all cases it was placed in a cold store at –7° C. within 24 hours of bleeding. The samples were removed from cold storage as required and on thawing no unusual odour or other signs of bacterial action was found. In some there were small fibrin clots which became dispersed after well shaking the bottles. The volume of each was measured and 200 ml. were removed, to be divided into 10 ml. quantities and stored in 20 ml. bottles at –5° C. until required. The remainder was converted into a stable gonadotrophic powder for subsequent biochemical work.

The biological activity of each sample was estimated by the ovarian weight method. Plasma, diluted when necessary, was injected into immature female rats (40 to 50 g.). Some received single injections ; others, multiple injections once daily for five days. All were killed 120 hours after the first injection. The ovaries were dissected out, fixed in Bouin's fluid for 24 hours, and dehydrated in 70 per cent. alcohol for two hours, and then weighed on a torsion balance. Parallel experiments were carried out using single or multiple doses of an international standard gonadotrophin, and of a subsidiary standard serum gonadotrophin. The log dose—log response curves depicted the activity of the plasma sample in international units/ml. As potency varied considerably, many preliminary tests had to be performed to determine the dose of plasma required for accurate assay, *i.e.*, to give paired ovary weights within the range 40 to 100 mg. The single injection method was used to save time, but multiple injections were necessary with several samples which were of low activity, many requiring doses up to 10 ml.

RESULTS

Diagnosis of Pregnancy

Measurements of the foetuses from A, B, C, D and F, as given in Table I, agreed closely with the results of rectal palpation in providing indication of the stage of pregnancy although the latter invariably indicated a shorter duration. The foaling date of mare G also verified substantially the estimate made by rectal examination in August, 1943. The foetal ages given in Tables II, III and IV and in the rest of this paper were estimated by rectal palpation. Limitations in accuracy of the method must therefore be borne in mind.

TABLE I
DETAILS OF MARES AND FOETUSES

	MARE			FOETUS					
No.	*Age*	*Approximate Weight*	*Estimated stage of pregnancy at death*	*Head Length mm.*	*Crown-Rump Length mm.*	*Height at Withers mm.*	*Eye Space mm.*	*Sex*	*Estimated age Days*
	Years	Lb.	Days						
A	2	450	82	55	148	82	26	Male	95–100
B	6	550	87	53	145	85	26	Male	95–100
C	8	550	82	47	135	75	20	Female	90–95
D	15–20	450	77	48	130	70	20	Female	90–95
E*	9	600	—	—	—	—	—	—	—
F	3	500	98	65	185	89	27·5	Female	100–110
G†	2	350	—	—	—	—	—	—	—

Activity of Plasma

Considerable differences (Tables II and III) were shown to occur in the activity of the plasma from mare to mare, in respect to (1) the maximum concentration of hormone attained; (2) the time when the maximum was attained; and (3) the period during which high concentrations lasted.

In mare C the peak value was rather low (73 IU/ml.); in the others it varied between 110 and 440 IU/ml. In five the maximum was reached between the 55th and 64th day, while in the others it occurred earlier (47th and 52nd day). As the blood samples were taken at *weekly* intervals, the true peak concentration may be somewhat higher than those recorded.

High levels were usually maintained for a short time only, the values falling rapidly after the 70th day (see Table II), but in one pony (G) a high level was determined for a long time, the value remaining at 118 IU/ml. on the 101st day. No explanation, clinical or otherwise, could be given of the sudden fall on the 55th day following the early peak concentration, in pony B, whereas with pony E the sharp decrease in concentration can be explained by the occurrence of abortion about the 60th day, an event which was quickly followed by diminution in the biological activity of the plasma. The low figures obtained in mare F after the early peak of 110 IU/ml. may possibly be explained by its poor physical condition. The rapid fall in serum gonadotrophin in several of the mares is

* Aborted at about the 60th day of pregnancy.
† Mare foaled 22.6.44. If gestation period was 340 days, stage of prgenancy at first bleeding was 40 days, cf. estimate of 50 days by rectal examination.

TABLE II

BLOOD AND PLASMA VOLUMES AND BIOLOGICAL ACTIVITY

Mare	*No. of Sample*	*Estimated Period of Pregnancy*	BLOOD *Volume (L)*	PLASMA *Volume (L)*	PLASMA *Biological Activity I.U./ml.*
A	A1	50	2·25	1·39	67
	A2	57	2·35	1·54	336
	A3	64	3·5†	2·24	440
	A4	71	2·5	1·76	224
	A5	78	2·5	1·72	26
	A6	82	11·0*	6·85	6
B	B1	55	2·25	1·29	200
	B2	62	2·45	1·48	68
	B3	69	4·5†	1·72	35
	B4	76	2·45	1·80	17
	B5	83	2·45	1·63	19
	B6	87	13·5 *	9·00	4
C	C1	45	2·25	1·11	54
	C2	52	2·25	1·47	73
	C3	59	2·4	1·60	39
	C4	66	4·5†	3·10	19
	C5	73	2·4	1·60	17
	C6	77	12·5*	8·20	6
D	D1	45	2·25	1·33	70
	D2	52	2·4	1·40	78
	D3	59	2·4	1·31	184
	D4	66	4·5†	3·16	124
	D5	73	2·4	1·66	96
	D6	77	11·0	7·56	31
E	E1	45	2·25	1·20	3
	E2	52	2·35‡	1·40	78
	E3	59	2·4	1·63	152
	E4	66	4·5†	3·22	4
	E5	73	3·75	2·66	3
	E6	77	2·5	1·71	1
F	F1	47	2·3	1·02	110
	F2	54	2·4	1·40	78
	F3	61	4·0†	2·61	17
	F4	68	2·4	1·68	13
	F5	75	2·25	1·48	6
	F6	88	2·8	1·50	2
	F7	93	9·5 *	6·45	2
G	G1	50	2·4	1·47	57
	G2	57	2·4	1·40	78
	G3	64	2·4	1·80	272
	G4	71	2·75§	1·86	200
	G5	78	3·0§	1·62	188
	G6	91	2·9§	1·61	130
	G7	101	1·0	0·50	118

* Mares bled out.

† Double quantities.

‡ The first bottle (about 2·1 L) was broken, *i.e.*, about 4·5 L were taken at this bleeding.

§ Because of the small size of the pony, extra quantities were taken in three successive bleedings, rather than a double quantity at one period.

TABLE III

MAXIMUM CONCENTRATION OF SERUM GONADOTROPHIN (IU./ML.)

Mare	*No. of Sample*	*Maximum Value IU/ml.*	*Estimated Stage of Pregnancy*
			Days
A	3	440	64
B	1	200	55
C	3	73	52
D	3	184	59
E	3	152	59
F	1	110	47
G	3	272	64

in accord with the results of Day and Rowlands (1940), who noted a more rapid rate of disappearance than was found by Cole (1938).

The above figures are in general agreement with those of other workers who have found variations of the same order. The data must be interpreted with due regard to the possible errors inherent in the work such as were likely to arise from the limited number of samples, the estimations of the stage of pregnancy and the method of biological assay.

Yields of Hormone from Individual Ponies

The total amount of hormone (international units—see Table II) obtained from each mare was calculated and the results as well as other relevant data are given in Table IV.

TABLE IV

YIELDS FROM INDIVIDUAL MARES

Mare	*Period of Experiment Days of Pregnancy*	BLOOD *Volume (L)*	PLASMA *Volume (L)*	PLASMA *Total Biological Activity I.U.*	PLASMA *Mean Biological Activity I.U./ml.*
A	50 – 82	24·10	15·50	2,076,000	134
B	55 – 87	27·60	16·92	516,000	31
C	45 – 77	26·30	17·08	363,000	21
D	45 – 77	24·95	16·42	1,229,000	75
E	45 – 77	17·75	11·82	383,000	33
F	47 – 93	25·65	16·14	312,000	19
G	50 – 101	16·85	10·26	1,627,000	158
A–G		163·20	104·14	6,506,000	63

Comparison of the yields with the weights of the ponies (see Table I) suggests that the best results both in regard to the highest

total activity and the highest mean activity were obtained from the three smallest ponies (G, A, D). When allowance is made for the smaller volume of blood taken from mare G, the best results were obtained from this animal, which was not only the smallest but, with A, the youngest. Too much stress must not be placed on the age factor because the oldest (pony D) also gave good yields.

Volume of Blood Samples

The regular withdrawal of large quantities of blood did not seem to have any pronounced effects either on the health of the animals, the concentration of the hormone or the duration of pregnancy, observations which are in agreement with those of Day and Rowlands (1940).

The blood volume of each pony was estimated assuming the ratio 10 L./100 kg. body weight (Dukes, 1942). It ranged accordingly from 16 L. (pony G, weight 350 lb.) to 27 L. (pony E, weight 600 lb.). Although the amount of blood taken each week was 2 L. or more, in only two cases (A3, F3) was bleeding stopped owing to signs of weakness, and on both occasions double quantities were being taken. The ponies became normal within half an hour. After withdrawing a double quantity, mare D (D4) went down on turning into the meadow, but got up and fed on approach.

Withdrawal of double quantities did not appear to affect the concentration-time curves. Table II shows that the extra quantities from all ponies were usually taken following the peak concentration, *i.e.*, at a time when the activity of the serum was normally decreasing, but in one pony (E) a double quantity was taken before the maximum. Here the almost filled bottle was kicked from the assistant's hand during the course of bleeding (E2) and another winchester was filled so that approximately 4·5 L. were withdrawn, yet the concentration at the next collection (E3) was considerably higher. Mare E, the largest of the group, aborted about the 60th day, but the others, from which a larger portion of the circulating blood was withdrawn, carried their foals. It is unlikely therefore that E's abortion was caused by excessive bleeding but rather to this animal being very wild and always strongly resisting the bleeding operations. Roughly one-half the total blood volume was estimated to have been obtained from the five ponies bled out. Jugular phlebitis was observed in D5 only. The vein of each of five ponies (A, B, C, D, F) dissected *post-mortem* showed some thickening of the walls although the lumen was of normal size.

Animal Husbandry

The ponies had come off the Welsh moors whereas throughout the experiment they were confined to a paddock, and were receiving hay, oats and bran. What effect the change of environment and extra feeding had on the concentration of the gonadotrophic hormone

could not be ascertained, but the general impression obtained was that the additional oats and bran were unnecessary. Regular bleeding should be possible without injurious effects if the ponies are kept on good pasture with a small hay ration and ample water.

Observations on Blood Samples

Although 25 ml. of 10 per cent. potassium oxalate placed in the sterile winchesters was usually found adequate, slight clots were formed occasionally, and so an extra 25 ml. of oxalate solution was tried in a few bleedings but without obvious advantage. There was no need for vigorous shaking during collection, mixing the contents well immediately afterwards being sufficient. The long coagulation time in the horse in part, at any rate, facilitated plasma collection. Amendt (1922) gives the coagulation time at 11½ minutes at 25° C.; Stewart and Holman (1940), using the capillary tube method, have recorded variations between 4·5 and 12 minutes with a mean of 8 to 9 minutes. The blood corpuscles sedimented very quickly, distinct separation being apparent within 5 minutes of withdrawal, and in 30 minutes sedimentation was almost complete, as was proved by marking the level on the bottle and comparing it with that seen 24 hours later. Providing winchesters or glass vessels of similar diameter are used for the collection of the blood, separation of the major portion of the plasma should be possible after a relatively short interval, *e.g.*, two hours, and the plasma placed in cold storage within three or four hours after bleeding, deterioration being thus reduced to a minimum. Accelerated sedimentation of red cells during pregnancy has been observed in woman [cf. Gradwohl, (1938)]; this may also play a part in the pregnant mare. The layer of white cells between the erythrocytes and the plasma was especially marked in the blood from animals bled out; the plasma layer in the bottles containing blood from the later stages of these bleedings, *i.e.*, after about 4 L. had been withdrawn, was very cloudy when first separated, resembling lipaemic plasma. The turbidity was attributed to the presence of large quantities of leucocytes and possibly platelets which settled after 24 hours to form a thick " buffy " layer. In the samples obtained under chloral anaesthesia (mare F) this was especially marked, and in addition it was noted that the plasma layer after 24 hours was coloured. Evidently the chloral had produced some haemolysis. The average yield of plasma was 64 per cent., 163 L. of blood giving 104 L. of plasma (see Tables II and IV). It is probable that only a slightly increased yield could have been obtained by the use of a centrifuge and any gain in this respect would be more than counterbalanced by the extra manipulations required to maintain sterility during centrifugation.

Several previous workers have used serum in preference to plasma, and either collected the blood in dry bottles without an anticoagulant or converted plasma from oxalated blood to serum by the addition of calcium chloride, followed by straining and Seitz

filtration. To minimise risk of loss of activity due to extra manipulation this procedure was omitted from our work. No disadvantage was found in the use of plasma either in the biological assays or in subsequent chemical work.

As noted earlier, the plasma kept well at –7° C., and although collection was carried out under field conditions the sterile precautions adopted were adequate. Plasma kept for nine months has shown no deterioration. The blood and plasma samples throughout the work were kept in *glass* vessels since previous experiments had indicated that loss of activity may occur in metal containers.

To investigate the possible effect of anaesthetics on the activity of the plasma a control sample of blood [F 7(a)] was taken immediately before chloral anaesthesia and its activity compared with the blood [F 7(b)] taken after the mare was anaesthetised, and injections of each plasma totalling 10 ml. were given over five days to each test rat. The F 7(a) group gave mean ovary weights of 21·0 mg. while the F 7(b) group gave 22·3 mg. as compared with normal ovary weights of 9 mg. We hesitate therefore to draw definite conclusions from this experiment for although both plasmas were definitely active the ovary weights in both cases were below the values required for accurate assays (40 mg. in our strain of rats). Chloral, however, in this experiment had little effect on depressing the activity of the plasma (estimated value 2 IU/ml.) which was already of low activity.

SUMMARY

Blood samples were taken from seven pregnant ponies at weekly intervals from about the 45th to the 80th day of pregnancy. Plasma was separated from the blood after 24 hours and stored at –7° C.

In five cases estimation of the stage of pregnancy by rectal examination was checked by comparison with that from foetal measurements and in one case from the date of foaling.

Biological assays of the gonadotrophic hormone were carried out on the plasma samples; maximum values were usually obtained between the 55th and 64th day, the peak concentrations varying between 73 and 440 IU/ml. High levels were maintained only for a short period.

In one mare which aborted the concentration dropped from 152 to 4 IU/ml. in one week.

An average yield of 64 per cent. plasma was obtained by the use of the siphoning technique; the rapid rate of sedimentation of the red cells in mares' blood suggests the practicability of separating the plasma two hours after the conclusion of the bleeding operations.

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OBSERVATIONS ON SUBLUXATION AND LUXATION OF THE CRYSTALLINE LENS IN THE DOG*

By

C. Formston,

Royal Veterinary College, London

INTRODUCTION

The prevailing view has long been, and still is, that luxation and subluxation of the lens in animals is commonly not hereditary and is produced by trauma to the lens or eyeball. Veterinary textbooks on ophthalmology either disregard or make scant mention of the disease, which is stated to be either congenital or acquired, and in the latter case to be usually the result of injury. The supposed infrequency of the disease is presumably to be explained away on these grounds, for at any rate evidence of hereditary instability in the lens of equines and bovines, among which groups the condition has most frequently been reported, has been lacking.

Because, however, of the frequency with which it has been seen in the Beaumont Animals' Hospital of the Royal Veterinary College, the disease seems certain to be more common in dogs than is generally supposed and in them it is almost restricted to a few breeds. The work detailed in the present paper was begun in 1937 and embodies some results of two years research into the problem of lenticular luxation and subluxation as it affects the dog.

LITERATURE

One of the earliest accounts of luxation of the lens in animals is provided by Nunn (1886). He records, without giving details, having seen the condition in the horse, mule and ox. Gray (1909), commenting on the incidence of the disease, concluded that it was encountered more frequently in the horse than the dog and cat. Twenty-three years later, however, his views (Gray, 1932) appear to have undergone a radical change for he now regarded the condition as essentially a disease of fox terriers, sealyhams and spaniels and estimated that when it occurred, in 95 per cent. of the cases it was bilateral. These observations, among the earliest recorded, support the theses presented in the present paper, namely, that the disease is hereditary and usually bilateral. Gray also reported hydrophthalmos in a dog resulting from lenticular displacement. McCunn (1934) suggests that malposition of the lens occurs as a frequent complication of glaucoma. The work of Wright (1934) remains, nevertheless, the most significant contribution upon the condition to veterinary literature, more especially because he has clearly appreciated the selective breed incidence, the disease according to him being predominant in the pure bred wire-haired fox terrier and sealyham.

* Part of a Thesis submitted for the Fellowship Diploma of the Royal College of Veterinary Surgeons.

MATERIAL AND METHODS

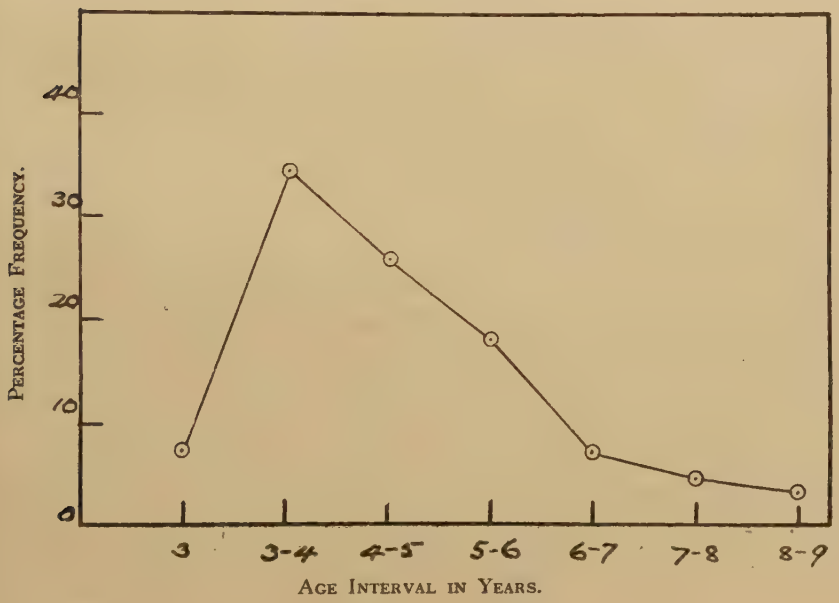
Observations were made on the course of the disease in over 100 cases and on eyes removed at operation or post-mortem and fixed. Full records were thus obtained from the examination of 160 eyes, while control observations were made on several normal eyes from wire-haired fox terriers and sealyhams.

As fixative 4 per cent. formalin was employed, which, after removal of the eye, was injected into the vitreous humor through the optic stalk. The pressure exerted was the most that could well be applied without interfering with the shape of the eyeball, escape of the fixing fluid being prevented by placing a ligature around the optic nerve beyond the point of entry of the hypodermic needle.

INCIDENCE OF THE DISEASE

Breed.—The disease has been encountered in only two breeds, namely, the wire-haired fox terrier and the sealyham, the animals affected having been good specimens of their respective breeds (see Table I). Of the cases examined, as shown in the table, 90 per cent. were wire-haired fox terriers and 10 per cent. sealyhams. The first of these breeds, it has to be noted, however, is by far the more popular breed with town dwellers. Wright (1934) gives figures that are in substantial agreement, namely, 80 per cent. wire-haired fox terriers and 15 per cent. sealyhams.

Text Fig. 1.



Age.—A striking feature is the relationship between age and incidence which has evident casual significance. No animal was seen affected under three years old. Wright (1937), however, has recorded a case of unilateral luxation in a two-year-old wire-haired fox terrier. From age distributions, presented graphically in text Fig. 1, lenticular displacement is seen to be most common between the ages of three and six years, the mode being four years.

TABLE I

WIRE-HAIRED FOX TERRIERS

Age in years	*Number of cases*	*Per-centages*	*Males*	*Females*	*Unilateral dis-placement*	*Bilaterial dis-placement*
3	7	7·7	4	3	5	2
3½	4	4·4	3	1	3	1
4	27	30·0	14	13	11	16
4½	5	5·5	3	2	1	4
5	18	20·0	6	12	7	11
5½	4	4·4	4	0	2	2
6	12	13 3	5	7	2	10
6½	3	3 3	2	1	0	3
7	3	3·3	2	1	1	2
8	4	4·4	3	1	2	2
9	3	3·3	3	0	2	1
Total	90		49	41	36	54
			SEALYHAM TERRIERS			
4	1		1	0	1	0
4½	1		0	1	0	1
5	3		3	0	0	3
5½	1		1	0	1	0
6	4		2	2	0	4
Total	10		7	3	2	8
Combined total	100		56	44	38	62

Sex.—Of the animals examined, 56 per cent. were males and 44 per cent. females, this disparity being perhaps unimportant, because males predominate as household pets. Moreover, the recorded frequency distribution among males and females in the different age groups (see Table I) does not indicate that such higher incidence among males is consistently maintained.

Unilateral and bilateral lesions.—Table I, which gives particulars of the affected animals when they were first presented, shows 62 per cent. of the cases to have been bilateral and 38 per cent. unilateral. As the author's experience has led him to believe that the disease is essentially bilateral, the percentage of unilateral cases recorded would seem at first sight not to support such a belief. The great majority of these cases, it has to be said, were diagnosed as

such during the early part of the investigation before the requisite experience was gained. The initial stages of luxation in the eye, thought then to be normal, could readily have been overlooked.

ETIOLOGY

General considerations.—The problem of etiology, on clinical grounds, appears to be identical in both breeds, but because more abundant data were forthcoming relating to the wire-haired fox terriers, discussion will be restricted here to this breed alone.

When dealing with affections of the special sense organs such as the eye, the impossibility of obtaining information from the patient as a subjective aid makes veterinary as opposed to human medical diagnosis particularly difficult. Information upon important symptoms, such as pain and disturbance of vision, upon which the ophthalmologist relies, is not forthcoming. Hence it is difficult to say with certainty whether the luxation is the primary condition, arising from disturbances confined to the lens and its ligament, or whether the luxation is secondary to some ocular disturbance arising elsewhere than in the lens.

Subluxation and luxation defined.—Luxation is taken to occur when the ligamentous attachment of the lens is completely disrupted and when it has vacated the fossa patellaris ; and subluxation when the ligamentous breakdown is partial and displacement incomplete. In eyes removed from the normal dog, irrespective of breed, the ligament is easily demonstrable post-mortem, either in the fresh or fixed eye, on applying pressure to the surface of the lens. It is a strong, resilient structure requiring considerable direct force to produce rupture of its fibres, thus contra indicating the frequent statements in the literature that trauma plays an important part in the etiology of the disease.

Trauma as an etiological factor.—The textbook generalisation that the disease may be either congenital or acquired, the latter resulting from trauma, is based on slight evidence. If trauma were commonly to result in the rupture of the zonule fibres, the disease should be met most often in breeds other than the wire-haired fox terrier and sealyham, but this does not appear to be so. The Pekingese, for example, which normally exhibits some exophthalmos, is particularly prone to varying degrees of injury and disease of the cornea, extending to deep ulceration and perforation of this structure and even to prolapse of the iris. Despite this, dislocation of the lens in this breed was not observed by the author and no record of its occurrence can be found in the literature.

Uberreiter (1937) admits that in most of his cases little or nothing could be found to support the " trauma theory." Atrophy of the suspensory ligament, he suggests, may be the result of a plastic irido-cyclitis and lens displacement take place subsequently. No inflammatory change could be seen in some of his cases, these suggesting to him hereditary weakness of the ligament or arrested development of the lens itself.

In the present series of cases trauma was thought to be a causative factor by the owners in only 10 per cent. A car accident was held to be responsible in one case, the dog, however, not being examined by us until one year after the accident, when it was then five and a half-years-old. In another case, a five and a half-year-old sealyham developed symptoms two weeks after being struck on the eye with a ball. A third dog, a four and a half-year old wire-haired fox terrier, showed defective vision immediately after it had been tossed by a pig. While trauma may be suspected in such cases as the cause, it is noteworthy that all (the above three being typical) were at the most susceptible age for lens displacement. While fighting with cats has been alleged as a cause, injuries to the cornea especially such as are caused by cats occur mostly in unsuspecting puppies, and in them, irrespective of the degree of injury and its spread to the uveal tract, the lens undergoes no displacement.

Wright (1934) pointed out that there was corneal opacity in all cases of lens luxation examined by him, which supported his view that trauma is the primary etiological factor. Wright's figures for the percentage showing corneal lesions are in substantial agreement with those recorded here, where out of 162 discernibly affected eyes as many as 141, or 87 per cent., exhibited some corneal change, varying from a slight localised opacity to a diffuse vascularised keratitis. Corneal opacity, however, accompanying lenticular displacement does not necessarily provide support for the trauma theory. The corneal lesions in the great majority occur as sequelae to the subluxation or luxation of the lens. Often the corneal lesion can be observed to have its inception in the posterior layers of the cornea and to be due to pressure from a partially or completely dislocated lens on these layers. Uberreiter (1937) has shown, moreover, that when an opacity is localised just below the centre of the cornea the anterior pole of the lens can be observed to lie in contact with its posterior surface. This observation has often been confirmed by us, and, furthermore, where hydrophthalmos was present the lens was most often found to adhere firmly to the cornea. The existence of a lesion of this kind in the cornea helps considerably in diagnosis. If not in itself diagnostic, along with other symptoms, it furnishes a useful clue to the real abnormality. Similar localised corneal lesions are also found associated with subluxation where the lens is tilted and its equator comes into contact with the posterior surface of the cornea.

Most of the corneal lesions, however, originate externally, affecting progressively the epithelium and subjacent corneal layers, and are caused by the affected animals blundering into obstacles because of the defective vision occurring after lens displacement. Since in this way injury becomes often repeated, the lesion tends to become progressive and develops into a chronic interstitial keratitis. That the ketatitis is secondary to the lens displacement is supported by the result of observations on 21 eyes in which no pathological change

had occurred in the cornea. In nearly all these eyes the displacement of the lens was in its early stages, not being sufficient as yet markedly to impair vision. These eyes appeared normal on casual observation but closer examination showed luxation to be imminent. A case in point was that of a six-year-old wire-haired fox terrier brought to the clinic for destruction because of chronic otitis externa. Close examination revealed early bilateral subluxation with no corneal involvement. When informed the owner volunteered that the dog for the past week or two had been very apprehensive and had exercised great care in walking, especially in strange surroundings.

Glaucoma and its relation to subluxation and luxation.—Partial or complete displacement is generally the precursor of a secondary glaucoma, but much depends upon the position of the lens. When it falls into the anterior chamber, whether a synechia is set up or not, it can readily increase intraocular tension. On the other hand, if it falls into the vitreous, it causes little or no rise in tension or increase in the size of the globe.

That glaucoma or hydrophthalmos is frequently a sequel to a luxated lens has been recognised by Wright (1934), Westhues (1937) and Uberreiter (1937). Indeed, Westhues stresses the need for early removal of such a lens, not to improve vision but to prevent the onset of glaucoma. Uberreiter (1939), investigated 16 cases of secondary glaucoma in dogs and found that in 13 it was caused by luxation of the lens into the anterior chamber. Nevertheless, both Westhues and Uberreiter suggest that isolated cases of lens luxation are caused by an initial glaucoma, but no evidence is adduced in support of this suggestion by either observer. Since glaucoma of secondary origin is known to occur in breeds other than wire-haired fox terriers and sealyhams, lens luxation ought, according to this view, to be seen in these other breeds. Secondary glaucoma is, in fact, frequently encountered in the Pekingese as a result of corneal perforation, with an anterior synechia, sometimes complete, but luxation or subluxation has never been observed in this breed. The present author is, therefore, unable to agree with McCunn (1934) that luxation is a frequent complication of glaucoma. Any theory that supposes lens luxation to be due exclusively to trauma or to be a frequent sequel of glaucoma must at present be regarded as inadequate; and any satisfactory theory of its origin must take into account the interaction of two sets of circumstances, namely, in the one case, those of a genetic kind, which in some unknown manner conduce towards disorganisation of the suspensory apparatus, and in the other, those of a non-genetic kind, as represented by the environmental conditions which are associated inseparably with such disorganisation.

Genetic basis of subluxation and luxation.—The data available from the present study, justify the belief that lenticular displacement as here encountered is a primary affection and is hereditable, that is, certain organic defects are inherited which terminate specifically in

a defective zonule. The cases under study seem peculiar only because of the delayed appearance of the defect, which becomes evident in early adult life, but this delay is by no means unknown to occur at the onset of several other hereditary defects that have been well studied. It is probably quite true that displacement of the lens is often precipitated by trauma, but before trauma can become effective some degree of genetic instability must, in the author's opinion, be present in the suspensory apparatus. That the disease is hereditable is presumed from the following circumstances :—

(1) The preponderance of bilateral affections which, as has already been shown, affects more than 60 per cent. of the cases. (2) The restricted and almost universal occurrence of the disease in two breeds of dogs only, namely, the wire-haired fox terrier and the sealyham. (3) The disease has been shown to be familial in several cases.

By the time the disease becomes recognisable clinically it is usually impossible to obtain a complete family history and so it has not been easy to obtain evidence in support of the last statement. The following three case records upon seven animals concerning which there could be no doubt about the authenticity of the family histories must, therefore, suffice :—

(1) A six-year-old wire-haired fox terrier and her four and a half-year-old son and daughter, all three of which were suffering from bilateral subluxation of the lens. The two offspring belonged to a litter of five, but all trace of the other litter mates had been lost. This case was interesting because the owner was not aware of the disease in the mother. Symptoms had been noticed, however, in the son and daughter simultaneously about ten days before examination. In both, secondary glaucoma developed rapidly and pain and interference with vision were so marked that within five weeks of the apparent onset of the disease the owner had both destroyed. It was otherwise in the mother's case, where the disease had not made appreciable progress three months after diagnosis.

(2) Two three and a half-year-old wire-haired fox terriers, being litter brothers. Both had been purchased at the same time and both had shown signs of defective eyesight within a fortnight of each other. One had complete luxation of the left lens which had passed into the anterior chamber, while the right eye at that time showed no detectable abnormality. The other was a typical case of bilateral subluxation.

(3) Mother and son, in this respect bearing some similarity to the first case alluded to. The mother, a seven-year-old wire-haired fox terrier, had had eye trouble for about two years. Examination of the eyes showed that both lenses were completely opaque and lying in the vitreous. The son, four years old, was a typical case of bilateral lens subluxation.

Ectopia lentis and a related syndrome in man.—Ectopia lentis, or lens displacement, in man is not uncommon and is invariably associated with a zonule which is poorly developed or entirely absent. Duke Elder (1938), in his review of the literature on the subject, regards this disease as hereditary and due to the presence of a

dominant " factor." Besides this congenital displacement, however, there occurs in man a spontaneous dislocation of the lens which is not traumatic in origin. It is this spontaneous dislocation which possesses features similar to those exhibited in the dog, in that displacement is bilateral and is not clinically demonstrable until adult life. The disease is frequently familial and many cases are recorded in the literature, the classical one being that reported by Vogt (1905). Here in three generations 16 people between the ages of 20 and 65 years developed the disease.

THE COURSE OF THE DISEASE

Pathological physiology.—Luxation of the lens is clearly due to instability and derangement of the suspensory apparatus. Normally the suspensory ligament is held in a state of tension by the intraocular pressure and only during accommodation is it relaxed. Irrespective of the degree of tension, however, and by implication the degree of curvature of the lens, the latter is held in such a position by the zonule that it always gives support to the posterior surface of the iris. The disease is presumably associated in its onset with degenerative changes, which are followed by stretching and ultimate rupture of the zonule fibres. As the fibres degenerate the lens ceases to be retained in a position in which it can properly support the iris. Consequently, with every movement of the head or eyes there is distinct tremor of the iris (Iridodonesis). This iridodonesis is symptomatic of the imminence of lens displacement. In the early stages of displacement, this may be the only symptom.

As the iris is extensively vascularised and richly innervated the exaggerated movement quickly gives rise to an iritis. By extending to the ciliary body the inflammation then produces an irido-cyclitis, often aggravated by the displaced lens acting as a foreign body, and it also causes a rise in the protein content of the aqueous humour. There is, in consequence, interference with filtration of this fluid and a resultant rise in intraocular tension (secondary glaucoma). This secondary glaucoma may be further accentuated by the increased pressure on the branches of the oculomotor nerve as they pierce the choroid to supply the sphincter iridis; this allows the antagonistic action of the dilator iridis which is supplied by the sympathetic, to be unopposed (Parsons, 1942). The resulting dilatation of the pupil further impedes the filtration of aqueous and contributes to the rise in intraocular tension. When the maximum dilatation is attained the oedematous iris tends mechanically to block the angle of filtration (namely, the point of confluence of the iris, ciliary body, sclera and cornea). Should the iris root make contact with the cornea, to produce a peripheral anterior synechia, the filtration angle is partially or completely eliminated. The secondary glaucoma affects the globe, a progressive and sometimes acute distension of the eyeball being an almost universal accompaniment of the rise in intraocular pressure. The increased size of the globe

contributes to the ultimate breakdown of the zonule and so, finally, the lens is luxated. Its escape from the fossa patellaris may, however, be postponed for some time, a circumstance apparently due to an inflammatory reaction, with adhesions, between the lens capsule and the hyaloid membrane. The final dislocation of the lens may sometimes be dependent upon disruption of the vitreous or the direct effect of some externally applied stimulus, when it may pass either into the anterior chamber or, more rarely, into the vitreous cavity. Whether the lens is subluxated or lies free in the anterior chamber, the enlargement of the eye continues and the lenticular displacement produces acute visual impairment. Injuries to the cornea are now more prone to occur. A keratitis develops, which is, at first, limited but after repeated injury becomes extensive. Deep vascularisation of the cornea then occurs, productive of a typical interstitial keratitis. The end result is a state of hydrophthalmos with the globe uniformly distended and the cornea flattened, opaque and invaded with scar tissue.

While the great majority pass through this phase of gradual zonular degeneration, a small minority do not appear to do so, when often, as the result of an accident, it is alleged that the lens is suddenly precipitated into the anterior chamber (see text Fig. 5). Only four such cases have been encountered in the present study. A state of hydrophthalmos is reached quickly in them, due in no small measure to the mechanical obstruction of the filtration angle by the dislocated lens itself. The absence of an hereditary defect in the suspensory apparatus in these acute cases is most unlikely as is, too, the probability that trauma alone would precipitate the condition.

CLINICAL MANIFESTATIONS

As patients are presented when the disease has reached different stages, the clinical manifestations may well be considered under the following headings : (1) Subluxation. (2) Luxation into the anterior chamber. (3) Luxation into the vitreous. (4) Hydrophthalmos.

SUBLUXATION

History.—Enquiry will elicit often in many cases a history of transient attacks of acute pain, blepharospasm, lachrymation lasting 24 to 36 hours, and, frequently, a hazy " blue film " over the cornea persisting sometimes for several days. The true nature of the lesion not being appreciated by the owner in such cases the patient may be treated for a " cold in the eye." Sometimes a trauma will be attributed to be the exciting cause but more frequently there is no definite evidence of recent injury. In the former case it may be ascribed to a blow from a ball, or the animal may be said to have fallen downstairs or to have been involved in other accidents. Although the acute symptoms mentioned tend to recur, they are transient and often overlooked so that it is seldom that an affected

dog is presented before there has accumulated distinct evidence of defective vision in one or both eyes. One may then learn that the dog has difficulty in following the flight of a ball or in retrieving it, that travel on stairs, even those to which it is accustomed, has become hazardous, or that the animal is loth to leave its home surroundings and often blunders into the household effects. This is more especially so if the disease is bilateral.

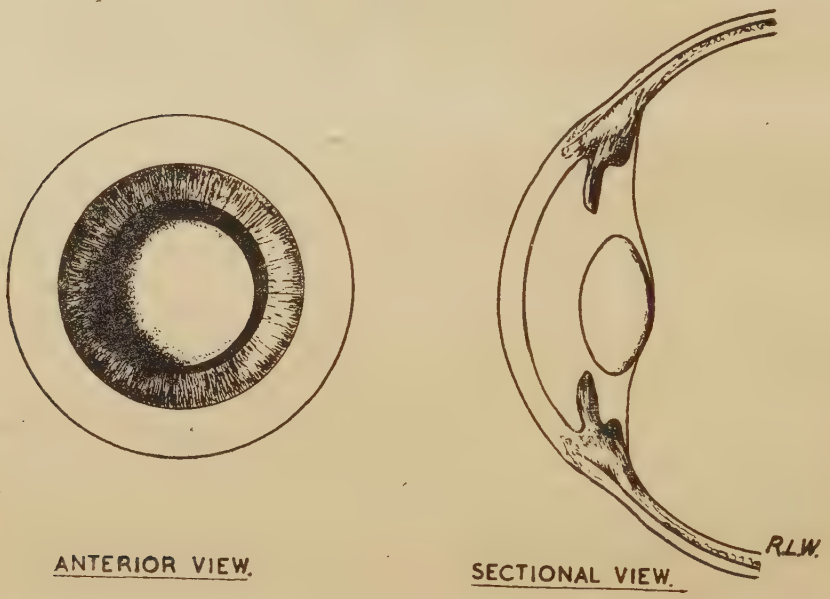
Examination and diagnosis.—Before examining the eyes the general behaviour of the dog should be observed. When affected it is generally apprehensive and nervous. Allowed freedom, it explores its new surroundings warily, and when objects are deliberately put in its path and more especially if it is in an unaccustomed environment, it may blunder into them or stop before them suddenly as though it has become conscious of them only on near approach.

When examining the eyes strong light is best avoided particularly in cases where photophobia is an accompanying symptom. The ideal place for examination is on a table near a window in good diffuse daylight. The dog must be restrained without interfering needlessly with its head. When it has become composed the eyes should be observed carefully as to size and any increase or disparity in size of the eyeballs noted. In unilateral cases of some duration proptosis of the affected eye may be obvious and in bilateral cases the eyes may be distinctly asymmetrical. The increased intraocular tension arising in the manner already explained and producing increased size of the eyeball with stretching of its tunics, may be gauged by applying digital pressure to the outer surface of the eyelid and, as already pointed out, it is highest during the initial stages of the disease and decreases proportionately with progressive ectasia of the sclera and distension of the eyeball. Accompanying this increase in tension there is intense congestion of the anterior ciliary and subscleral vessels.

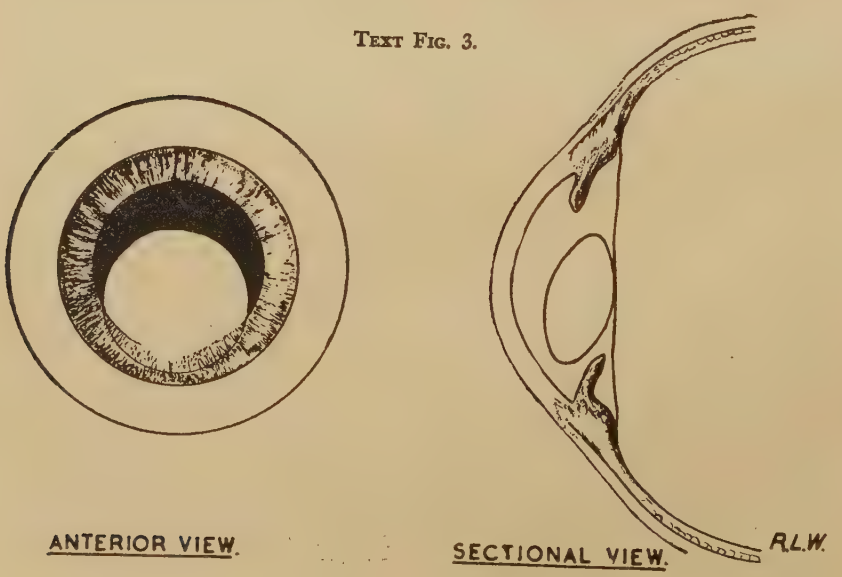
The cornea may be transparent, or it may show localised opacity or diffuse haziness. When injury to the cornea is recent and its epithelium has been abraded, there is generally an associated photophobia and corneal oedema, but corneal vascularisation is not often encountered in cases of subluxation, and consequently in these cases there is no impediment to investigation of the deeper structures of the eye. When the iris comes under observation it will be noticed that its reaction to light is sluggish, the pupil in most cases being dilated and incapable of maximum constriction. The depth of the anterior chamber will then usually be seen to be shallower than normal because increased intraocular pressure tends to push the iris forward. While the iris remains capable of movement, iridodonesis is constant. When the pupil becomes dilated to its maximum a part or the whole of the equatorial edge of the lens becomes visible. It will then be seen that, no matter whether the zonule is partially or completely ruptured, the lens may either still be placed more or less centrally in its fossa or else have gravitated ventrally into the

posterior chamber or towards the inner canthus. Thus, the pupillary aperture will have become, in some part at least, what is termed aphakic (that is, the crystalline lens will be absent from it). If the lens is still in its central position this aphakic area will be ring-like (see text Fig. 2). On the other hand, with translocation of the lens, it becomes crescentic in outline (see text Figs. 3 and 4). In either

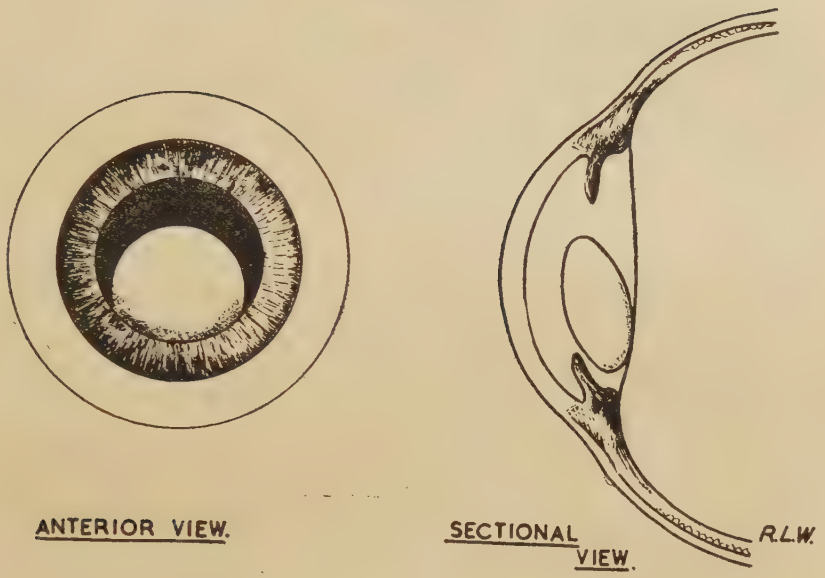
Text Fig. 2.



Text Fig. 3.



Text Fig. 4.



case the appearance of this aphakic area is characteristic since it is more luminous than the rest of the pupillary opening, which is attributable to the intensity of a pencil of light reflected from the tapetum lucidum in this region not being diminished by having to pass back through the lens. This phenomenon becomes accentuated with focal illumination and the lens then appears grey, its free edge being identifiable as a light grey line to which the remains of the suspensory ligament may still be attached in the form of radial striations. When the eye is examined with an ophthalmoscope, however, the lens takes on a golden or golden green hue and the aphakic area becomes dark grey. The lens will often be found to have undergone not only ventral translocation but also to have rotated on its transverse axis so that a segment enters the anterior chamber while the rest remains in the posterior chamber (see text Figs. 3 and 4) where it is retained generally because of adhesions between the posterior surface of the lens capsule and the hyaloid membrane bounding the vitreous. To a lesser extent, adhesions to the iris hinder complete liberation of the lens in these cases. The pupillary zone of aphakia again becomes crescentic in shape when the lens resumes this position of transverse rotation, and should the greater part of it come to occupy the anterior chamber, then it may be caused to oscillate with movements of the head.

Examination of the optic disc is made difficult by voluntary eye movements and by errors of refraction arising from rotation of the lens, but when the lens is centrally placed the difficulties are less serious. For the inspection of the vitreous and fundus, the ophthal-

moscope is essential and although the examination can be made in ordinary light it is best conducted in the dark room. Rarely are pathological changes observed in either the retina or vitreous. Floating opacities are occasionally seen in the vitreous but their significance remains unexplained. The venous circle of the optic disc and its retinal branches generally appear normal and on no occasion has " cupping " of the optic disc been observed. The absence of this latter phenomenon, which is diagnostic of glaucoma in man, is due, no doubt, to distension of the eyeball occurring before intraocular pressure is raised sufficiently to indent the lamina cribrosa. Optic atrophy as revealed by a pale avascular disc has been encountered in only two cases of lens subluxation although it has frequently been seen in post-distemper blindness and blindness of unknown origin.

Diffuse corneal opacities offer the only real obstacle to the diagnosis of subluxation. Nevertheless an opacity, when present, will be found most marked at the centre of the cornea and progressively less marked towards the periphery, while at the limbus, where lymph circulation is but little impaired, the cornea is clear. In these cases examination is facilitated by using focal illumination.

The diagnostic features of a subluxated lens may be summarised accordingly as follows :—

(1) Increased intraocular tension associated in its train with distension of the eyeball and scleral conjunctival congestion.

(2) A dilated pupil ; an iris which reacts sluggishly to light ; iridodonesis.

(3) A zone of pupillary aphakia with the edge of the lens partly or wholly visible.

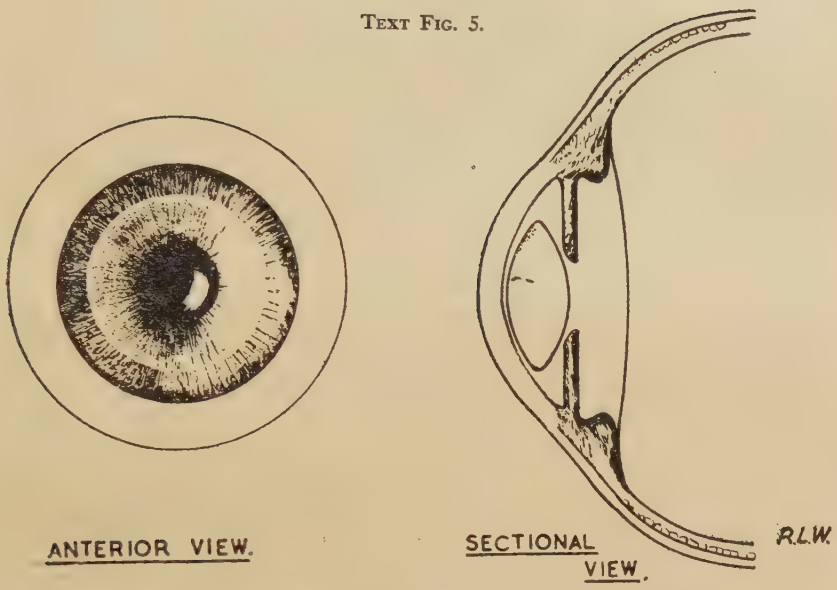
LUXATION OF THE LENS INTO THE ANTERIOR CHAMBER (ANTERIOR LUXATION)

This may be acute or, more frequently, subacute. In acute cases the lens is suddenly projected into the anterior chamber without having passed through an initial phase of subluxation, whilst in subacute cases it undergoes the preliminaries of a subluxation. Only a small percentage of cases are presented in the acute form.

Examination and Diagnosis.—Provided the animal is seen within a few days diagnosis is not difficult, but the position of the lens cannot be ascertained easily because of its transparency. Diagnosis is based principally on the marked tremor and spasm of the iris which is active and still capable of maximum construction. The position of the lens can be more easily determined by focal illumination, the light when concentrated around the corneo-sclerotic junction revealing the periphery of the lens as a silver grey line (see text Fig. 5). Should the lens move in the anterior chamber its position will be immediately evident and will confirm completely the diagnosis.

When luxation has existed for some time there is obvious increase in intraocular tension with inhibition of the sphincter iridis and

TEXT FIG. 5.



dilatation of the pupil. Distension of the eyeball may be conspicuous and there will be marked congestion of the anterior ciliary and subscleral veins. The symptoms are now indistinguishable from those of the subacute condition. At this stage defects of vision are acute, and repeated injuries to the cornea produce well-defined inflammatory changes manifested as local and diffuse opacities. There is usually a superficial keratitis with vascularisation. This inflammation of the cornea adds to the difficulty of locating the lens but since the latter has by now undergone degenerative changes and become shrunken and opaque, its detection is to some extent easier. Furthermore, the adherence of its anterior pole to the posterior surface of the cornea produces a sub-central opacity which, as already indicated, is almost pathognomonic.

LUXATION OF THE LENS INTO THE VITREOUS CAVITY (POSTERIOR LUXATION)

The incidence of luxation into the vitreous is low and constitutes no more than 5 per cent. of the cases seen.

Examination and diagnosis.—The lens lying horizontally on the floor of the vitreous chamber does not interfere mechanically with the angle of filtration as does a lens free in the anterior chamber; nor does it act as a constant irritant to the iris as does a subluxated lens. Accordingly the prospects of hydrophthalmos arising are remote. The degree of astigmatism being minimal probably accounts for the relative infrequency of corneal injuries and opacities. In one case in which there was slight ocular distension there was present a sub-central corneal opacity suggestive of an anterior luxation, and it is possible that for a time the lens may have rested

in the anterior chamber before passing into the vitreous cavity.

In all cases of posterior luxation the cornea is sufficiently transparent to permit examination of the deeper structure. The eyeball is usually only slightly enlarged while the congestion of the subscleral vessels is only just perceptible. The pupil is only partially dilated. The iris is tremulous and the pupillary aperture totally aphakic. The lens is globular, opaque and easily recognised. The retina and optic disc are invariably normal.

HYDROPHTHALMOS

The term hydrophthalmos (buphthalmos) is used in human ophthalmology to designate infantile glaucoma, the main characteristic of which is gross distension of the eyeball. This is similar to what develops in dogs as the end result of a secondary glaucoma. It is difficult to state what stage of secondary glaucoma must be reached before the corresponding term hydrophthalmos is applicable, but, speaking generally, hydrophthalmos may be said to be present when distension has risen to a degree in which the eyeball cannot be enveloped completely by the eyelids.

All cases of hydrophthalmos in wire-haired and sealyham terriers, with the possible exception of one case in which no trace of a lens could be found post-mortem, have been associated with luxation of the lens into the anterior chamber. In the exceptional case it may be presumed, however, that a displaced lens was primarily responsible for its production.

Examination and diagnosis.—There is no difficulty in recognising hydrophthalmos, but owing to changes in the cornea it is often impossible to examine during life the interior of an eye affected with it. The eyeball completely fills the orbital cavity and its movements are restricted. There is marked proptosis and, in consequence, incomplete closure of the palpebral aperture. The cornea is flattened peripherally from pressure of the eyelids and bulges centrally. Continuous exposure often results in a drying keratitis of this central part which may then become anaesthetic and in the remaining cornea there is usually a diffuse interstitial keratitis. The scleral conjunctiva is congested. The most characteristic feature, however, is the blue colouration of the sclera in the ciliary region, which is actually due to the uveal pigment showing through the ectatic sclera. Ectasia of the sclera occurs in all cases, consequently intra-ocular tension is low and may even be less than normal. Reduced intra-ocular pressure may also arise from atrophy of the ciliary body, the secretory power of which may be diminished or even abolished. In all cases of hydrophthalmos blindness is inevitable, yet it is remarkable that in most cases the affected dog appears to be almost free from pain. Often a positive diagnosis of lens luxation cannot be made during life, nevertheless its existence may be inferred when all the collateral evidence is taken into consideration.

Post-mortem examination.—Gross destructive changes involving all or most of the contents of the eyeball are constant. The lens,

shrunken and opaque, is invariably found adherent to the posterior surface of the cornea. In many cases a limited portion of the iris is also adherent to the cornea while its pupillary margin is attached to the lens, thus completely obliterating the anterior chamber. The weakest part of the sclera appears to be situated between the ciliary body and the root of the iris. Ectasia and bulging of this region is common, giving rise to an intercalary staphyloma over which are stretched remnants of uveal tissue. Atrophy of the ciliary body is complete. The posterior and vitreous chambers are confluent, and contain a fluid similar to aqueous humour but often containing precipitates. The retina may be torn and partial or complete detachment may have occurred in long standing cases.

SUMMARY

More than 100 dogs affected with displacement of the crystalline lens have been examined.

The disease is essentially bilateral and has been encountered only in the wire-haired fox terrier and the sealyham terrier; it has not been observed in any animal under three years of age.

The primary aetiological factor is genetic but actual manifestation of the disease appears to be determined by environmental influences, principally trauma.

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LEGENDS TO FIGURES

FIGS. 1 AND 2.—Sagittal sections of normal eye of wire-haired fox terrier, three years (X. 2). Fig. 1 shows lens in position ; in Fig. 2 the lens has been removed. Note particularly the shape and position of lens (L.), position, size and shape of iris (I.) and ciliary body (C.B.) ; depth of anterior chamber (A.C.) ; the normal filtration angle (F.A.) ; the corneo-sclerotic junction (C.S.J.).

FIGS. 3 AND 4.—Frontal section of normal eye of wire-haired fox terrier, five years (x. 2·5). Fig. 3 shows anterior view with cornea removed. Note that with dilation of the pupil the iris (I.) still embraces the lens (L.) and obscures its periphery. Fig. 4 is a posterior view of the same eye as seen in Fig. 3. The plane of section passes posteriorly to the ciliary body (C.B.). Note the intimate contact of the ciliary body with the periphery of the lens. The zonular fibres are identifiable as light striations on the surface of the ciliary body.

FIG. 5.—Frontal section of right eye of female wire-haired fox terrier, four years (x. 2·5). Anterior view with cornea removed. Note dilated pupil, narrow crescent of aphakia (P.A.), and slight ventral displacement of lens (L.).

FIG. 6.—Posterior view of same eye as shown in Fig. 5 ; plane of section passes posteriorly to ciliary body. Note stretched and ruptured zonule fibres (Z.F.).

FIG. 7.—Frontal section of right eye of female wire-haired fox terrier, four years (x. 2·3). Anterior view with cornea removed. Note the ventral displacement of the lens (L.) ; the dilatation of the pupil ; the localised posterior synechia (P.S.) ; the fibrinous exudate (F.E.) adherent to lens and iris (I.) and the irregularity of the pupillary margin.

FIG. 8.—Oblique frontal section of the same eye as shown in Fig. 7. Plane of section passes posteriorly to ciliary body. Note almost complete detachment of retina (R.) except at point of entry of optic nerve (O.N.). This detachment exposes the choroid (C.H.).

FIG. 9.—Frontal section of right eye of male wire-haired fox terrier, five years (× 2·1). Anterior view with cornea removed. Note recession of the lens (L.) into the vitreous cavity ; a posterior synechia (P.S.) and a wide crescent of pupillary aphakia (P.A.).

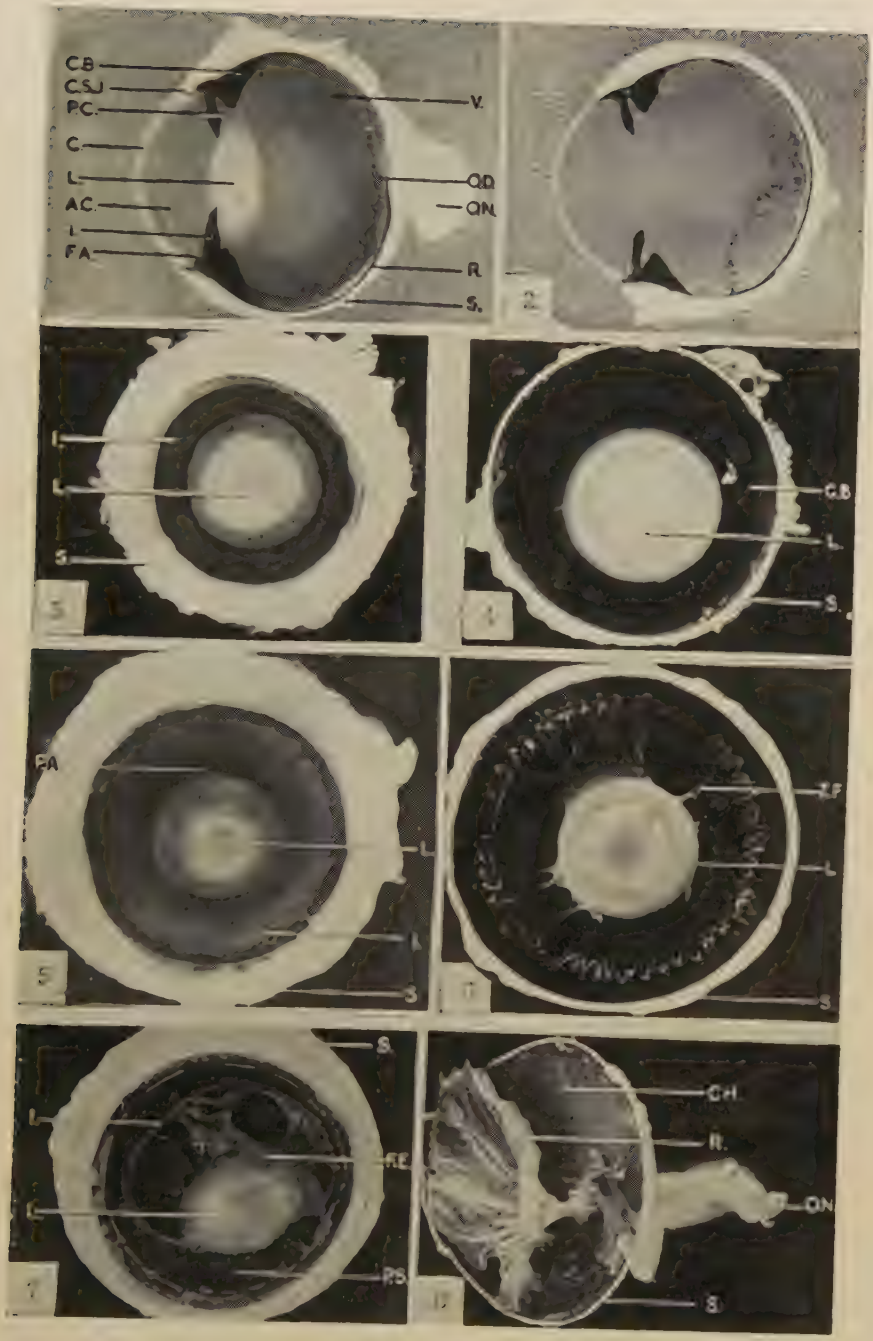
FIG. 10.—Sagittal section of right eye of female wire-haired fox terrier, five years (× 2). Lens dislodged. Note peripheral anterior synechia (P.A.S.) with occlusion of filtration angle ; shallowness of anterior chamber (A.C.) ; the atrophic ciliary body (C.B.) and the incipient intercalary staphyloma (I.S.).

FIGS. 11 AND 12.—Sagittal sections of right eye of male wire-haired fox terrier, five and a half years (× 1·6). Note shape and position of the lens ; adhesion of iris to cornea ; ectasia of the sclera in the region of the ciliary body—intercalary staphyloma (I.S.). The light striations in Fig. 10 indicate the zone where the light has penetrated the stretched sclera ; the dark striations constitute strands of uveal pigment stretching over the ectatic sclera between the ciliary body and the root of the iris.

FIG. 13.—Right eye of male wire-haired fox terrier, nine years (× 2·5). Frontal section through the globe with component parts still partly attached. The plane of section passes posteriorly to the limbus. Note that the lens (L.) is adherent to the cornea and that the iris (I.) forms a ring synechia with the lens. The lighter striations (L.S.) indicate the zone where the light has penetrated the stretched sclera. The dark striation (U.P.) represents strands of uveal pigment bridging the ectatic sclera.

List of Abbreviations

A.C. = Anterior Chamber
C. = Cornea
C.B. = Ciliary Body
C.H. = Choroid
C.S.J. = Corneo-Scleral Junction
F.A. = Filtration Angle
F.E. = Fibrinous Exudate
I. = Iris
I.S. = Intercalary Staphyloma
L. = Lens
O.D. = Optic Disc
P.A. = Pupillary Aphakia
P.A.S. = Peripheral Anterior Synechia
P.C. = Posterior Chamber
P.S. = Posterior Synechia
R. = Retina
S. = Sclera
S.S. = Scleral Staphyloma
S.T. = Sealyham Terrier
U.P. = Uveal Pigment
V. = Vitreous
W.H.F.T. = Wire-haired Fox Terrier
Z.F. = Zonule Fibres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C.B.
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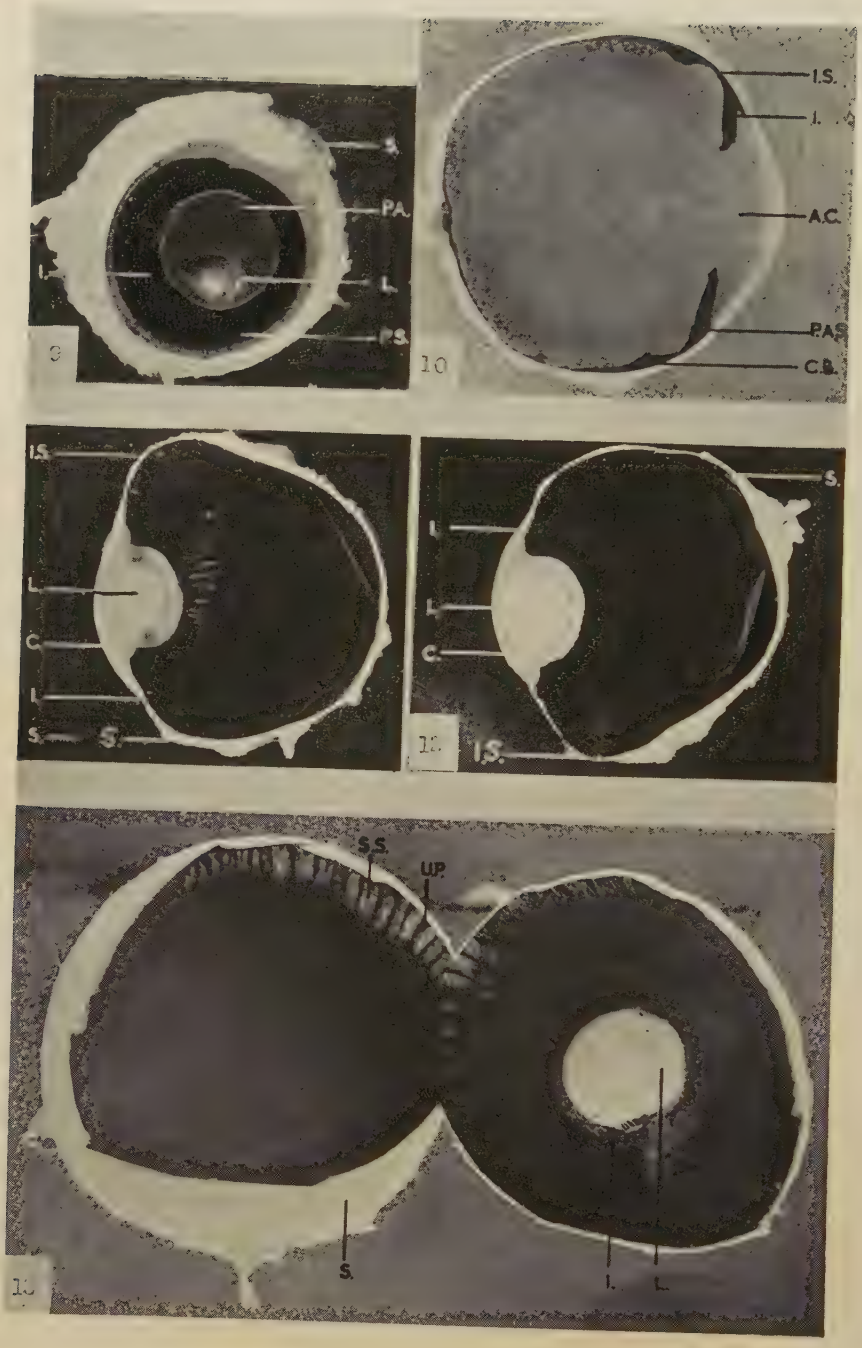
Figs. 1 to 8.



Figs. 9 to 13.

OBSERVATIONS ON RINDERPEST IN GOATS IMPORTED TO MALAYA

By

William Orr,

Senior Veterinary Officer, Palestine, Sometime Veterinary Officer, Singapore

INTRODUCTION

In 1939, following the outbreak of war, circumstances necessitated the importation of large numbers of goats to Malaya from India to meet military requirements. The importation of cattle, sheep and goats from India had been prohibited unconditionally by the Malayan Governments for some years before 1939 owing to the danger of introducing disease—in particular rinderpest. After a prolonged campaign, the Malayan Veterinary Service ultimately succeeded in eradicating enzootic rinderpest from Malaya and for nearly a decade only relatively small outbreaks of this disease occurred in the Straits Settlements and Federated Malay States directly traceable, in almost every instance, to the introduction of infected animals from territories where it was known to be enzootic. After 1935 these outbreaks were restricted entirely to imported stock undergoing quarantine at the ports of entry. Having regard to its geographical position and the prevalence of the disease in many other Far Eastern countries, British Malaya was exceptionally fortunate latterly in its freedom from rinderpest. The maintenance of this state of affairs, however, depended on the constant vigilance of the Veterinary Departments, particularly in regard to imported stock.

The susceptibility of Malayan oxen and buffaloes to rinderpest was known to vary widely according to breed and variety. The typical Indian zebu variety of ox and the curly horned Indian dairy buffalo exhibited a relatively high level of resistance to infection while the indigenous wide-horned water buffalo of Malaya was liable to contract the disease in a peracute, rapidly fatal form. Observations on the disease in Malaya showed that infection was liable to spread insidiously and unobtrusively among the resistant types of cattle until it reached highly susceptible stock such as the water buffaloes, when its progress became subject to sudden change, the disease then assuming all the characteristics of a rapidly spreading epizootic causing heavy mortality. The intermingling of resistant stock and of highly susceptible stock within an infected area therefore tends to increase the practical difficulties of control and eradication of the disease.

Other difficulties confronting the veterinarian in controlling rinderpest in the field in Malaya are described by Turner (1930) and by Sheedy (1932). The latter referring to an outbreak which occurred in Selangor in 1931, writes: "If rinderpest should again gain entrance there is little doubt that the same difficulties would be encountered, the same obstinate neglect

of, or systematic opposition to elementary precautions, the same cunning dodging of quarantine regulations, the same degree of non-co-operation in combative measures and the same losses inflicted on the few helpful and amenable owners through the obstructive, or equally undesirable defeatist attitude of the majority."

In 1941, when several shipments of goats arrived in Singapore infected with rinderpest, the situation was complicated by the presence of large numbers of imported Bali oxen, which were known to be extremely susceptible to rinderpest.* Government at this time had embarked upon a scheme for the purchase of Bali oxen with the object of maintaining some thousands of these animals as a meat reserve on the hoof in Malaya. The cattle were imported for purchase in Singapore and from there distributed throughout the various States and Settlements for maintenance. The import traffic in slaughter stock to Singapore was thus enormously increased, resulting in serious overcrowding and overlapping of consignments in the veterinary quarantine stations on the island. Infection of the imported Bali oxen in these circumstances would have been immediately disastrous and the organisation for importation, purchase and distribution of the cattle reserve would have been brought to a standstill.

The drastic measures, described hereunder, which were adopted in dealing with infected shipments of goats, were based on the following considerations :—

(*a*) Malaya at the time was entirely free from rinderpest.

(*b*) Introduction of infection among local livestock could be expected to incur heavy losses before the disease could be eradicated. This conclusion was based on experience gained in former outbreaks in Malaya to which brief reference has already been made.

(*c*) Heavy losses could be expected if infection spread to the highly susceptible Bali cattle overcrowded in Singapore quarantine stations.

(*d*) The important scheme for building up a food reserve of cattle would be held up indefinitely in the event of contingency (*c*).

(*e*) Under war-time conditions the conservation of existing local food stocks was of major importance. An extensive destructive epizootic was liable, in the circumstances, to assume more than economic importance.

HISTORY OF RINDERPEST IN IMPORTED GOATS IN SINGAPORE, 1941

During June, 1941, four shipments of Indian goats arrived in Singapore from Calcutta, and in three of them rinderpest was diagnosed on arrival of the ship in port, considerable mortality

* The Bali Ox, or banteng, is believed to belong to a practically pure race of cattle which has evolved during domestication from a wild species of gaur or seladang (*Bos gaurus sondaicus*). Though widespread outbreaks of rinderpest have occurred in Java, Sumatra and other Islands of the Netherlands East Indies, the disease has never been known to occur in the island of Bali. As a result of observations made on a number of occasions in Malaya, the banteng was known to be extremely susceptible to rinderpest.

being reported to have occurred during the voyage, while in the fourth, transhipped at Port Swettenham, on the west coast of the Peninsula, no definite rinderpest was detected on arrival in Singapore and only two deaths had occurred during the voyage. On the following day the first case was diagnosed.

These goats were imported by a military contractor in Malaya to whom a special permit had been issued by the Veterinary Authority. They were intended solely to supply the Military Authorities. Three of the four consignments were accompanied by " Certificates of Health " issued in Calcutta, in which it was stated that a specified number of goats, intended for shipment to Malaya, had been examined and that they " appeared to be in a state of health and no evidence of disease of an infectious or contagious nature was present." In the case of the shipment which was transhipped in Port Swettenham no certificate was produced.

Shipment A arrived in Singapore on June 4th, when several goats were found to be severely affected, eight dying during the afternoon. Out of 500 shipped in Calcutta, 40 were reported to have died during the voyage. A tentative diagnosis of rinderpest was made and arrangements were immediately concluded to isolate the surviving goats in army premises on Pulau Blakang Mati, an island separated from Singapore by a narrow strait. On June 5th, after detailed clinical and *post-mortem* examinations, rinderpest was diagnosed. The disease continued to spread rapidly and cause heavy mortality during the eight days after arrival of the shipment in Singapore until the slaughter of the survivors, on June 12th. These were killed after consultation with the military authorities so that the premises on Blakang Mati might be cleared of infected

SHIPMENT A

Arrived Singapore	4.6.41
Number shipped in Calcutta	500
Number arrived in Singapore	460
Number died on voyage	40

Rate of Mortality in Singapore

June	*Died*	*Destroyed*	*Balance*
4	8	—	452
5	15	—	437
6	16	—	421
7	24	9	388
8	13	6	369
9	29	7	333
10	17	6	310
11	19	4	287
12	50	{ 65 172(A) }	—

(A) = Apparently healthy contacts slaughtered on 12.6.41

animals and disinfected before the arrival of the next consignment of goats from India. Of these slaughtered survivors 65 showed obvious clinical infection. Detailed *post-mortem* examinations of all goats slaughtered could not be carried out owing to the numbers involved and the need for haste in the disposal of carcases. The following table indicates the incidence and death-rate, the animals shown as " destroyed " between June 7th and 11th, inclusive, having been destroyed *in extremis*.

Shipment B arrived in Singapore on June 12th, when several goats were found to be in an advanced stage of the disease, 22 dying within a few hours of their arrival in port. Out of 500 goats shipped in Calcutta, 72 were reported to have died during the voyage. Heavy weather had been encountered during the passage from India which may have contributed to the heavy mortality. There was no doubt, however, that the mortality in this shipment after arrival in Singapore was due to rinderpest. The surviving goats were discharged directly into lighters and conveyed to Blakang Mati, where the disease spread rapidly among them. The daily mortality rate was heavier than in Shipment A. The survivors of Shipment B, 61 in number, were slaughtered on the morning of June 20th, within eight days after arrival in Singapore, when nearly all of them were exhibiting severe rinderpest; it was then concluded that the mortality would have approached 100 per cent. if the outbreak had been allowed to run its course. The following table indicates the incidence and death-rate. The animals shown as " destroyed " between June 12th and 19th inclusive, were, again, destroyed *in extremis*.

SHIPMENT B

Arrived in Singapore	12.6.41
Number shipped in Calcutta	500
Number arrived in Singapore	428
Number died on voyage	72

Rate of Mortality in Singapore

June	*Died*	*Destroyed*	*Balance*
12	24	1	403
13	31	13	359
14	31	19	309
15	47	—	262
16	62	—	200
17	45	27	128
18	20	24	84
19	12	11	61
20	—	61(A)	—

(A) = Majority severely affected

Shipment C arrived about June 13th in Port Swettenham, where the animals were transhipped and sent on by sea to Singapore. At Port Swettenham they were examined by the veterinary authority, who reported that all were apparently healthy. On arrival in Singapore on June 16th two goats appeared somewhat dull, but thorough clinical examination failed to reveal any further symptoms suggestive of rinderpest. Out of 200 shipped in Calcutta only two were reported to have died during the voyage. From previous experience of this traffic it was estimated that a mortality of 1 per cent. could be regarded as normal transit wastage. Thus, on the evidence then available, it appeared probable that this shipment was not infected with rinderpest. The premises on Blakang Mati were at the time occupied by the infected goats of Shipment B. It was decided, therefore, to accommodate the apparently healthy goats of Shipment C in an improvised quarantine station on Singapore Island under close observation.

Next day (June 17th) one of the above two goats developed diarrhoea and died in the late afternoon. Symptoms suggestive of rinderpest were seen in a second goat, which was immediately slaughtered. The tentative clinical diagnosis of rinderpest was confirmed *post-mortem* in both. Before the survivors of this shipment were destroyed, on June 18th, six died and one was slaughtered in an advanced stage of disease. In all, 26 exhibited clinical symptoms and showed characteristic rinderpest lesions *post-mortem*. From the ample clinical and pathological material available for examination, the diagnosis of rinderpest could be readily based—a point that needs to be stressed, since at the time of the outbreak, and afterwards, doubts were expressed as to the validity of the diagnosis in this particular shipment. These doubts arose from the fact that in each of the other infected shipments there had been heavy mortality during the voyage from India and the disease was readily detected in several goats on arrival in Singapore. Besides, rinderpest was most unlikely to have " smouldered," it was contended, under the conditions of close contact on shipboard and to flare up suddenly, affecting 26 animals within 48 hours of landing in Singapore.

The shipment was never at any time, during or subsequent to landing, in direct or indirect contact with infected animals, premises or areas in Singapore. The first case to be detected was in one of the two goats known to be sick on arrival in port. This animal, therefore, must have contracted the disease before arrival. The possibility of the infection having been contracted in Malaya can therefore be dismissed. The apparent latency of infection and its delayed manifestation among the goats of Shipment C cannot be explained. The circumstances, nevertheless, have practical implications in providing adequate quarantine periods for goats imported from infected countries.

The potential danger of a focus of infection actually on the Island of Singapore was fully appreciated by the civil administration,

and authority was obtained for the disposal of the whole shipment by slaughter and burial in lime. This was carried out on June 18th. The following table indicates the incidence and death-rate :—

SHIPMENT C

Arrival in Singapore	16.6.41
Number shipped in Calcutta	200
Number arrived in Singapore	198
Number died on voyage	2

Rate of Mortality in Singapore

June	*Died*	*Destroyed*	*Balance*
16	—	—	198
17	1	1	196
18	5	{ 19(A) 172(B) }	—

(A) = Clinically infected animals.
(B) = Apparently healthy animals.

Shipment D arrived in Singapore on June 20th, when several goats were found in an advanced stage of rinderpest, five dying within a few hours. Out of 300 shipped in Calcutta, 16 were reported to have died during the voyage. About 50 exhibited clinical symptoms of rinderpest on arrival in port. The shipment was discharged direct into lighters and taken to Pulau Terkukor, an uninhabited islet south of Blakang Mati, where they were slaughtered on June 21st. The following table indicates the incidence and death-rate :—

SHIPMENT D

Arrival in Singapore	20.6.41
Number shipped in Calcutta	300
Number arrived in Singapore	284
Number died on voyage	16

Rate of Mortality in Singapore

June	*Died*	*Destroyed*	*Balance*
20	5	—	279
21	13	{ 37(A) 229(B) }	—

(A) = Infected animals.
(B) = Apparently healthy animals.

HISTORY OF RINDERPEST IN IMPORTED GOATS IN PENANG, 1941

During June, 1941, in addition to the Indian goats imported to Singapore, a smaller number were imported to Penang. The

goats for Singapore and Penang were drawn from the same consignments, gathered together in Calcutta, awaiting shipment. The goats consigned to Penang, as was to be expected, also arrived in Malaya infected with rinderpest. The disease manifestations were similar to those observed and recorded in the goats imported to Singapore during the same period. As in Singapore the disease was successfully eradicated by slaughter of all the imported goats.

In Penang, experiments were conducted with the object of determining the susceptibility of Bali oxen and local goats to rinderpest virus after natural passage through the imported Indian goats.* The virus, in these experiments, proved to be highly pathogenic to both species of experimental animal. The peracute rapidly fatal reactions that were evoked in Bali oxen were essentially similar to those observed in these animals following infection with rinderpest virus of bovine origin as described by Whitworth and Rocker (1936) and by Rocker (1939). As a result of these experiments any doubts concerning the identity of the disease were disposed of conclusively. The extreme susceptibility of Bali oxen and local goats to the Indian goat virus served to emphasise further the necessity for the eradication of the disease by the most effective means, which was the elimination of all actual and potential foci of infection. This could only be achieved, in the circumstances, by the slaughter of all the imported goats involved.

It was decided in June to discontinue the importation of Indian goats to Malaya pending the provision of more adequate quarantine accommodation and a revision of the conditions under which the traffic might be resumed. Shipment D, which arrived in Singapore on June 20th, was the last importation for some months. The outbreak of hostilities in Malaya in December, 1941, prevented the resumption of importation except on a limited scale. There was no recurrence of rinderpest.

SYMPTOMS AND LESIONS OF RINDERPEST OBSERVED IN IMPORTED GOATS IN SINGAPORE, 1941

Symptoms

The initial symptoms in affected goats were depression, capricious appetite and thirst, followed by anorexia, dyspnoea, harsh staring coat and the adoption of postures indicative of severe abdominal pain. In the majority of cases diarrhoea occurred as an early symptom while in a few a period of marked constipation persisted for some time after the onset of premonitory symptoms. The typical initial changes in the buccal mucosa were generally apparent early in the course of the infection, though in many cases it appeared that a fatal termination was reached before the processes of epithelial dissolution and ulceration reached an advanced stage. The buccal lesions

* The Penang experiments were conducted by Mr. W. R. Wallace, M.R.C.V.S., D.T.V.M., of the Malayan Veterinary Service.

conjunction with severe gastro-intestinal disturbance, as evidenced by continuous, often haemorrhagic, diarrhoea, and the high rate of mortality were the most outstanding features in these outbreaks and those upon which the initial tentative diagnosis of rinderpest was based pending confirmatory *post-mortem* evidence. The disease ran an acute or peracute course, the period from the onset of symptoms until death varying from about 24 hours to three or four days. During the short periods that infected consignments were under observation there was no evidence that recovery ever occurred in infected goats. It is probable that had a slaughter policy not been adopted as a control measure, a few goats might have proved resistant to infection or might have recovered. From the observations which it was possible to make, however, it was evident that the percentage of survivors would have been extremely low.

The nose became dry and the mucosa markedly inflamed at an early stage in the disease and in a number of cases punctate haemorrhages developed. A copious clear watery discharge tended to drip at frequent intervals from the nares when the head was lowered. On occasion the discharge was markedly frothy. In some cases ulceration of the mucosa and profuse encrustration of the nostrils with inspissated muco-purulent discharges occurred. The condition appeared to cause considerable irritation as evidenced by violent movements of the head, frequent sneezing and occasional rubbing of the nostrils against inanimate objects or against the fore-limbs. (Nasal discharges and coughs are of common occurrence in goats and the symptoms and lesions described could not in all cases be ascribed to rinderpest). Partial or total occlusion of the nostrils sometimes occurred giving rise to gasping oral respirations. Dyspnoea occurred as an early symptom and persisted throughout the course of the disease, stertor being marked in the later stages.

The eyes were kept half-closed, or closed, when the affected animal was not disturbed ; the conjunctivae were injected ; excessive lachrymation becoming muco-purulent was usual and the behaviour often suggested some degree of photophobia. The sunken appearance of the eye was in many cases emphasised by oedematous swelling of the eyelids and periorbital region. (Irido-cyclitis, corneal opacity, vesiculation and ulceration, lesions typically associated with malignant catarrh, were not observed.)

The buccal mucosa at an early stage in the disease became dry and inflamed, losing its normal, moist, glistening appearance. Minute pin-point elevations, generally grouped in clumps, appeared on the mucosa of the lips, increased in numbers and gradually becoming confluent involved the whole surface of the affected mucous membrane. In the latter a process of liquefaction and dissolution progressed rapidly, exposing the subjacent tissues to erosion and ulceration. In advanced cases, as a result of pathological changes in the epithelium, the mucous membrane of the mouth was covered with a loosely adherent deposit of grey or greyish-yellow, caseous,

mushy matter having a typical foetid odour. [This condition was described by Robertson (1924) as " branny " mouth.] Ulceration tended to be localised and discrete, the commonest site being the lips, tongue, hard palate, gums and dental pad. In severe cases, however, desquamation of the buccal epithelium was succeeded by the formation of extensive areas of ulceration on the raw exposed surfaces of the lips, gums, dental pad, hard palate and tongue, particularly the free extremity of the latter. In peracute cases death sometimes occurred before the characteristic epithelial changes became apparent or when such changes were only observed in their initial stages as minute localised inflammations affecting the extremities of a few buccal papillae. The mouth lesions were therefore regarded to some extent as an indication of the severity of infection. In the more chronic cases the ulcerated areas tended to become deeper and more extensive and it appeared probable that secondary bacterial infection played a considerable part in the exacerbation of these lesions in their later stages of development. Cases of contagious ecthyma in which the lesions involve the buccal mucous membrane, when seen at the early vesicular stage, may give rise to a suspicion of rinderpest since, in the former disease the mucous membrane is dry, inflamed, reddened and marked by minute elevations (Orr, 1938). With the development of the disease and the emergence of the typical ecthymatous lesions the superficial similarity to rinderpest is lost. As contagious ecthyma is a prevalent infection among goats under local conditions (and to a lesser extent among sheep) its transient resemblance to an early phase of rinderpest is of practical significance. The mouth lesions of rinderpest when fully developed were typical and distinctive enough to be regarded, for practical purposes of diagnosis, as pathognomonic of the disease particularly when seen in numbers of animals. Excessive salivation occurred, fluid dripping from the lips. Unlike the typical oral discharge seen in cases of foot-and-mouth disease the fluid was not ropy. It was in many cases mixed with air bubbles, becoming very frothy. Frequent exaggerated swallowing movements were observed as if to clear the pharynx of accumulated fluid and the tongue was frequently protruded and retracted.

Similar oral symptoms and lesions were recorded by Beaton (1930) in his observations on rinderpest in Nigerian goats. He stated, however, that these lesions did not become apparent in the infected goat before the tenth day at the earliest, whereas in the Indian goats seen in Singapore (as in cattle) the lesions occurred at an early stage in the course of the disease. Stirling (1933), in his account of rinderpest in goats in India, makes no mention of mouth lesions which, together with diarrhoea, were the most prominent diagnostic symptoms in the Singapore cases. Mouth lesions were also observed in the majority of affected goats in the outbreak of undiagnosed disease which occurred in Singapore in 1935 as described in a later section of this paper.

Severe diarrhoea appeared to be a constant symptom, though it was frequently not apparent for some time after the emergence of general febrile symptoms. In the earlier stages diarrhoea was usually profuse. Intermittent tenesmus was marked and accompanied by evidence of acute pain. The fluid faeces were forcibly expelled following these frequent seizures. In the terminal stages tenesmus was less marked and the diarrhoea tended to become uncontrolled, fluid dribbling more or less continuously from the partially prolapsed anus. The fluid faecal matter, which was generally dark in colour and of very offensive odour, was not infrequently admixed with mucus, undigested food particles, and blood. Considerable clots of the latter were passed in some cases. The blood content of the faeces was, on occasion, arterial in appearance but more frequently dark and partially decomposed. Occasionally the evacuation appeared to consist almost entirely of fluid with practically no solid matter in suspension. The quarters were badly soiled and the perineum severely " scalded " and inflamed through continuous contact with irritant faecal matter.

With the onset of symptoms affected animals were, as a rule, dull, disinclined to move and prone to stand in dark corners with the head turned away from the light. Other animals appeared to be indifferent to their surroundings and made no effort to seek shade during the hottest times of day. In a few cases the occurrence of profuse diarrhoea preceded the onset of general febrile symptoms and animals, though scouring badly, appeared to be quite bright.

Affected goats were markedly " tucked up " with the back arched, the hind limbs well forward under the body and the head and neck lowered. The latter were not infrequently rested upon the edges of feeding troughs or on irregularities in the walls of the pen. There were frequent indications of severe pain as evidenced by grinding of the teeth, grunting, stamping of the forefeet, restlessness, frequent turning of the head to the belly and flank and erratic, apparently aimless progression. Weakness, particularly of the hindquarters, was progressively marked; spastic cramps, possibly consequent upon superpurgation, gave rise to a paraplegia-like condition. Incoordination of movement was generally followed by complete loss of control of the limbs and collapse with the limbs splayed out before and behind in grotesque unnatural postures. In some cases frenzied ineffectual attempts were made to rise from the recumbent position. The hindquarters might then be raised and when collapse occurred the animal would, as a rule, pitch forward with the neck twisted laterally. In a number of cases there appeared to be a total or partial loss of voice due probably to an extension of the mouth lesions to the larynx. In such cases attempts to bleat or cry out resulted in hoarse expiratory gasping. Throughout its course from the onset of general symptoms until the fatal termination the disease was characterised by severe pain, the indications of which were abundantly evident particularly in the later stages. Neither stupor

nor coma intervened to relieve the acute suffering which appeared to reach its greatest intensity immediately before death. During the latter stages of the disease affected animals made a peculiar expiratory moaning. When numbers of animals in a flock were affected the volume of sound was so considerable as to be audible at some distance.

During the initial febrile phase the pulse was large, later becoming small and almost imperceptible. Fairly marked jugular pulsation was observed in a number of cases. Some time before death occurred there was a tendency for the extremities and, to a lesser extent, the body to become cold. The mouth and muzzle at this stage were distinctly cold and clammy.

Both dysuria and incontinence of urine were observed in affected animals.

Lesions

The most severe typical and constant lesions were observed in the alimentary tract and to a lesser extent in the gall bladder. Tracheitis, bronchitis and pneumonic changes occurred but were so variable and inconstant in their manifestations that these conditions were regarded, in a proportion of cases at least, as intercurrent infections exacerbated in all probability by rinderpest. According to Beaton's observations, pulmonary complications in rinderpest were more common in goats than cattle. He observed that, in the goat, fatal reactions in experimental infections were characterised by a broncho-pneumonia which may or may not have been a specific symptom of rinderpest. The disease in Nigerian goats, where not complicated by pneumonia, was usually mild and non-fatal. In this latter respect the Singapore cases differed widely, many fatalities occurring without significant pulmonary complications.

The mouth lesions have been described in some detail in the preceding section. These lesions affecting the hard palate frequently merged posteriorly with a severe inflammatory condition involving the soft palate. The pharynx was frequently deeply congested and inflamed. Liquefaction necrosis of the pharyngeal mucosa, however, was generally patchy or absent; small areas of ulceration were observed in a number of cases. It was not uncommon, however, to find no gross pathological changes in the pharyngeal cavity.

Constant pathological changes were not observed in the oesophagus. Congestion, catarrh and patchy haemorrhagic inflammation were seen in some cases. No changes of any significance were observed in the rumen, reticulum and omasum, though in the last named there was a tendency for the contents to be of an abnormally fluid consistency. The abomasum was the seat of fairly constant, and within a certain range, typical lesions. In the most characteristic cases the mucosa had a purple-brown colouration, the surface being marked by punctate haemorrhages, localised areas of epithelial erosion and discrete ulcerations, these being covered by adherent,

yellow, scab-like, croupous deposits varying in size from a minute point to about 2 cm. in diameter. As a rule inflammatory changes were most marked in the pyloric part. The *plicae spirales* appeared swollen and oedematous. Extensive ulcers with elevated edges occurred in some of the more chronic cases. In one autopsy deep ulceration involving actual perforation was seen. Where extensive submucosal ecchymoses occurred haematomata of considerable size sometimes developed. The contents of the organ were fluid in consistency, of a sour putrescent odour and, as a rule, dark in colour, with an admixture of mucus shreds and blood in various stages of decomposition. Small quantities of undigested food were present.

The small intestine was catarrhal and its wall thickened and oedematous. Extensive desquamation of the mucous membrane resulted in the formation of loosely adherent, fragmentary, decomposing, mucous casts. In some cases haemorrhagic inflammation occurred involving the whole, or large portions, of the small intestine. Submucosal petechiae were common throughout the length of the bowel. The contents were fluid and resembled those of the abomasum. The ileo-caecal valve was a constant site of acute haemorrhagic inflammation, the submucosa appearing to be markedly swollen and engorged with blood. The most severe lesions in the alimentary canal were commonly encountered in the caecum, the thickened walls of which were inflamed and intensely haemorrhagic. Catarrhal inflammation with profuse desquamation and extensive ulceration, the formation of diphtheritic pseudo-membranes, ecchymosis, copious extravasion of blood into the caecal lumen, and, in occasional cases, gangrene of the apical portion of the organ were among the pathological changes observed. Large blood coagula were present in the lumen where severe haemorrhage had occurred. Changes in the large intestine were as a rule most marked posteriorly where they culminated in severe haemorrhagic inflammation of the anal mucosa. The mucosa of the whole organ, as a rule, tended to be catarrhal. Haemorrhagic inflammation was typically localised, assuming a longitudinal linear disposition following the folds of the membrane and not infrequently marked by numerous petechial haemorrhages. Erosion and ulceration of the partially prolapsed anus was common.

The mesenteric and other associated lymph glands were enlarged and frequently haemorrhagic on section.

Slight congestion of the liver was observed in some cases but no typical or relatively constant lesions occurred in this organ. Characteristic lesions not infrequently occurred in the gall bladder, upon the mucous membrane of which minute scabs, tending to coalesce in groups, were seen. Erosions also occurred and the bile contained quantities of semi-solid " mushy " material which appeared to be cell detritus from the diseased mucosa.

The kidneys, in most cases examined, showed fairly well marked congestion of the cortex.

Inflammatory changes involving the pharyngeal mucosa as a

rule extended to the larynx and in some cases to the trachea. The respiratory passage from bronchi to larynx was not infrequently filled with finely churned yellow froth sometimes tinged red with blood. The commonest pathological change in the lung was an interstitial emphysema. Catarrhal pneumonia of very variable extent and severity occurred. Croupous pneumonia, on the other hand, was of rarer occurrence and was generally attributed to secondary causes. The lesions of the respiratory organs were of little diagnostic significance since they so closely resembled those seen frequently in association with mortality of obscure origin among goats kept under local conditions.

The heart muscle was in many cases abnormally flabby. Both endocardial and epicardial petechiae were observed.

The thoracic and abdominal cavities frequently contained a quantity of transudate sometimes blood-tinged. Slight hydropericarditis was also observed.

In striking contrast to the usual descriptions of the typical rinderpest carcase indications of excessive emaciation were often not apparent. On opening the abdominal cavity peritoneal fat was generally present in considerable amount and there was little indication of wastage of subcutaneous fat.

HISTORY OF THE OCCURRENCE OF AN UNDIAGNOSED DISEASE IN IMPORTED GOATS IN SINGAPORE, 1935

Whitworth (1936) gives the following account of an undiagnosed contagious disease of Indian goats imported to Singapore in October, 1935 :—

" Two consignments totalling 387 goats and 12 sheep were landed from Calcutta and placed under observation. Numbers of goats in each consignment were found to be sick on arrival. The symptoms noted included fever, diarrhoea, dysentery, nasal catarrh, conjunctivitis, dyspnoea of varying severity, cough, congestion of the buccal mucosa (usually followed by ulceration), and emaciation. A number of pregnant goats aborted. Sheep were not affected. A total of over 60 goats manifested combinations of the above symptoms. Nine goats, including two kids born in the quarantine station, died. The remainder recovered and were slaughtered in due course.

" *Post-mortem* examination of the fatal cases revealed congestion, inflammation, and ulceration of the mucosae of the abomasum and intestines ; pneumonic lesions of varying severity but not of great extent ; congestion, and frequently erosion of the lining of the gall-bladder ; cardial petechiae ; and congestion of the kidneys. . . .

" Some of these imported Indian goats were affected with what appeared to be a mild or ' chronic ' form of contagious pleuro-pneumonia. Others bore lesions of goat pox. It was considered, however, that these two affections were recognisable as entities distinct from the pneumo-enteritis causing severe symptoms and deaths. But this could not be said with certainty without further detailed work. Rinderpest infection was suspected and in order to confirm, or disprove, this suspicion, a series of contact and subinoculation experiments with local goats and with oxen and buffaloes was undertaken. The results were inconclusive and, unfortunately, rinderpest virus and facilities for cross-immunity tests were unobtainable at the

time. It is to be noted, however, that highly susceptible cattle, *e.g.*, Bali cattle did not react severely to inoculation with blood from sick goats. It was demonstrated that the disease was transmissible to local Singapore goats by subinoculation of blood and by direct contact. An experiment to test the susceptibility of Australian sheep was inconclusive. Subinoculation of blood into calves and oxen was usually followed by a reaction manifested in slight fever, diarrhoea, loss of appetite, congestion, followed by erosion, of the buccal and nasal mucosae, and conjunctivitis—a reaction, in fact, that resembled a very mild rinderpest reaction."

The most striking differences between the undiagnosed disease of 1935 and the rinderpest outbreak of 1941 appear to depend upon the relative degrees of virulence exhibited in the two outbreaks. The symptoms and lesions are essentially similar except in respect of their relative severity. Where differences appear to exist they may be attributed to the greater virulence of the disease in the later outbreak. Emaciation, for example, recorded as typical in the 1935 cases was not marked in the majority of cases in 1941 due, it may be assumed, to the rapidly fatal course of the disease. The virulence, symptoms and pathological changes in the 1935 outbreak in many respects resembled rinderpest as described in Nigerian goats by Beaton (1930), and to a lesser extent the disease in Indian goats as described by Stirling (1933). According to these authors the reactions among infected goats are relatively mild. The mortality among goats used for virus production is stated by Stirling to be about 15 per cent. In this respect the outbreak of rinderpest in 1941 with its extreme virulence and high rate of mortality differed widely from rinderpest as it has been generally described in goats in Africa, India and elsewhere. The undiagnosed disease of 1935, in fact, resembled the generally accepted picture of rinderpest in goats more closely than the confirmed outbreak of rinderpest in 1941. The differences which exist, therefore, between the outbreak of 1935 and that of 1941 comprise those atypical characteristics which in the case of the latter render the outbreak widely different from the accepted picture of rinderpest in the goat (Hutyra, Marek and Manninger, 1938). The heavy mortality experienced in 1941, though exceptional, is not, however, without parallel in the literature relating to rinderpest in the goat. Pfaff (1938) quotes D'Costa and Balwant Singh (1933), who state that in their experience the mortality in Indian goats may vary from 70 to 100 per cent., an estimate which approximates to the mortality which occurred in the outbreak of 1941.

In 1935, doubts as to the identity of the disease seem to have arisen chiefly from the indefinite reactions obtained following the attempted experimental transmission of the disease to cattle, and in particular to Bali oxen which from previous experience were known to be extremely susceptible to rinderpest. It was expected that the reaction of the Bali oxen would be severe. The typical reactions, however, which occurred in all the experimental oxen, not excepting the Bali oxen, actually resembled " a very mild rinderpest reaction."

It is now suggested that the mild nature of these reactions may be attributable to previous repeated natural passage of the virus in goats and its consequent lowered virulence for cattle. (Stirling describes the symptoms evoked in cattle by a goat passaged virus as sometimes so mild as to produce no noticeable symptoms.) The fact that the virus obtained from goats in the 1941 outbreak was extremely virulent for cattle is not as contradictory as it might appear in this connection since the rinderpest virus is not invariably attenuated for cattle as a result of passage in goats. Stirling made the observation that the virus, in some cases, became attenuated for cattle following initial passages in the goat while on subsequent passages in that species the virulence for cattle was enhanced. It follows that the effect on the virus of natural passage in goats of various breeds and origins is not constant or predictable.

The failure of an experimental buffalo calf to react visibly may have been due to the collection of the inoculum from the donor animal at a stage when the blood of the latter was no longer infective as suggested by Whitworth and Rocker. On the other hand, Singapore-bred buffaloes of this variety are known to exhibit a high level of natural resistance to strains of rinderpest known to be lethal for oxen (Rocker, 1939). There are therefore two acceptable explanations of the apparent absence of a reaction in the single buffalo used in these experiments.

The three Malayan bred goats used in these experiments contracted a disease which was indistinguishable from rinderpest in a severe form and two of them died.

No conclusive evidence can be drawn from these experiments upon which a definite diagnosis of rinderpest could be established. Such evidence as there is, however, does not preclude, in any particular, the possibility of such a diagnosis but on the contrary would appear in every respect to support it.

SUMMARY AND CONCLUSIONS

The general position with regard to the occurrence of rinderpest in Malaya in the past is briefly reviewed. No serious major outbreak had occurred for some years. There had been occasional localised occurrences due to the introduction of infection from other countries.

Malaya was free from rinderpest in 1939 when military requirements made it necessary to relax veterinary quarantine restrictions and to permit the importation of goats from India. The potential risks of introducing disease to Malaya with these animals were fully realised. Certain varieties of livestock in Malaya (*i.e.*, the indigenous wide-horned water buffalo and the imported Bali ox) were known to be extremely susceptible to rinderpest. When shipments of rinderpest-infected goats arrived in Singapore in June, 1941, the latter was overcrowded with numerous Bali oxen that were being imported in large numbers to supplement local food stocks. The consequences of an outbreak affecting indigenous stock and imported Bali cattle at that time would have been disastrous. When

the disease made its appearance in imported goats, therefore, it was imperative that the most effective measures should be enforced to eradicate infection without regard to the immediate losses incurred in the slaughter and disposal of the animals in the shipments involved.

An account is given in some detail of the occurrence of rinderpest in four infected shipments comprising in all 1,500 Indian goats that arrived in Singapore. The disease occurred in a highly infective and (for goats) unusually virulent form causing heavy mortality. In one of the four shipments only, rinderpest was not diagnosed immediately on arrival in the port. In this shipment, however, the disease was diagnosed within 24 hours of landing and within 48 hours was rapidly assuming the epizootic form which characterised the disease during these outbreaks.

A brief account is given of concurrent outbreaks of rinderpest in Indian goats imported to Penang. These goats were drawn from the same source in Calcutta as the infected shipments that arrived in Singapore during the same period.

Reference is made to experiments carried out in Penang from the results of which it was concluded that the rinderpest virus from imported Indian goats was highly infectious and virulent when transmitted to Bali oxen and locally bred Malayan goats. The reactions of the former animals resembled those evoked by a virulent virus of bovine origin.

The symptoms and lesions of rinderpest observed in goats imported to Singapore are described in detail.

Reference is made to Whitworth's account of an undiagnosed disease which occurred among Indian goats imported to Singapore in 1935. This disease was suspected at the time of its occurrence to be rinderpest. Accounts of the undiagnosed disease are compared.

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AVIAN POX IN WILD SPARROWS

I.—A NOTE ON A SPONTANEOUS OUTBREAK

By

C. A. McGaughey,

Dept. Bacteriology and Preventive Medicine, Manchester

II.—A NOTE ON THE ACTIVITY OF SPARROW POX VIRUS IN THE CANARY

By

F. M. Burnet,

National Institute for Medical Research, Hampstead

INTRODUCTION

Avian pox occurs commonly in fowls, turkeys, guinea-fowl and pigeons, more rarely in ducks, geese, pheasants, quail and hawks (Brunett, 1943). It has been described in buntings (Shattock, 1898), canaries (Eberbeck and Kayser, 1932; Reis and Norbrega, 1937; Grosso and Prieto, 1939; Antoniotto and Romat, 1940). Shattock (1898) mentioned that he had observed avian pox in sparrows. Experimentally, Lahaye (1930), Eberbeck and Kayser (1932) and Burnet (1933) succeeded in transmitting canary pox to sparrows. Irons (1934) studied a strain of pigeon pox which was pathogenic to English sparrows, ground sparrows, snow buntings and canaries; he suggested that this strain may have originated in sparrows but was unable to detect avian pox in numerous trapped sparrows and starlings.

According to Lahaye (1930) there are three types of avian pox virus: (*a*) fowl pox pathogenic to fowls, turkeys and guinea-fowl but not to pigeons nor to passerine birds; (*b*) pigeon-pox pathogenic to pigeons and transmissible to fowls but not to passerine birds; (*c*) canary or passerine pox transmissible to canaries, finches and sparrows but not to pigeons or to fowls. Eberbeck and Kayser (1932) accept this classification but recognise " bipathogenic " strains which they suggest may be really mixtures of strains. With their canary-pox strain they obtained on fowls and pigeons non-specific lesions, *i.e.*, on histological examination Bollinger bodies could not be detected; moreover, the lesions soon healed and the birds did not develop immunity to fowl and pigeon pox. Burnet (1933) showed that canary pox (Kikuth strain) was not pathogenic for day-old chicks, half-grown fowls, pigeons and budgerigars inoculated intramuscularly with highly active filtrates. Sparrows, however, were susceptible to infection. Later, Burnet and Lush (1936) showed that fowl pox and canary pox, although not identical, are serologically very closely related. Irons (1934) suggests that some strains of avian pox may be rendered " bipathogenic " by passage through a series of heterologous hosts, *e.g.*, fowl pox passaged through pigeons. The strain of canary pox studied by Reis and Norbrega (1937) was " tripathogenic " as it infected chicks, pigeons and passerine birds; typical inclusion bodies were found in infected birds and those which recovered developed immunity against fowl and pigeon pox; conversely, birds vaccinated with either fowl pox or pigeon pox developed immunity against this strain of canary pox.

On the other hand, the strain of canary pox investigated by Grosso and

Prieto (1939) produced only transitory localised lesions in chicks and pigeons and that studied by Antoniotto and Romat (1940) was pathogenic only to canaries.

As, apparently, there is no recent record of spontaneous infection in the wild sparrow, it is thought that the following note may be worthy of record. Some experiments in transmission of the virus to domestic fowls and pigeons were made; when further work was impossible for various reasons, some of the original virus which had been preserved in 50 per cent. glycerol saline for 15 weeks was sent to Dr. F. M. Burnet, then at the National Institute for Medical Research, Hampstead.

I.—ISOLATION OF VIRUS

Three sparrows were received at the Department of Bacteriology and Preventive Medicine, Manchester, at intervals during a period of eleven days in the spring of 1933; they had been found dead on a poultry farm in North Wales and forwarded to Mr. H. Britten, Assistant Curator at the Manchester Museum, who examined them for ectoparasites. Mr. Britten reported that two of the sparrows were infested with the poultry flea *Ceratophyllus gallinae Schrank*, the red mite *Dermanyssus gallinae Redi*, and the mallophagan louse *Philopteris communis*; on the third sparrow, lice and feather mites only were found. Mr. Britten remarked that in his experience wild birds were seldom infested with the poultry flea or red mite.

The pox lesions were apparent in the form of dry, tough, blackish crusts at the angles of the mouth and between the branches of the mandible; there were similar but smaller crusts on the skin of the neck, thorax and sternum in one of the birds. On *post-mortem* examination, the lungs were seen to be congested and the intestine slightly inflamed but no pathogenic bacteria or protozoa could be found.

The crusts from the first case were triturated with sand in a mortar and suspended in nutrient broth; some of this suspension was mixed with 50 per cent. glycerol saline and kept at 4° C.; the remainder was rubbed on the scarified skin and combs of two young cockerels. After three days typical but rather benign lesions appeared with the formation of fine crusts at the end of six days; one cockerel was killed and the suspension of triturated crusts applied to the scarified skin on the legs of two further young cockerels. Again, rather benign lesions appeared and soon healed.

The crusts from the second and third sparrows were similarly triturated, suspended in nutrient broth and inoculated on the scarified skin of a pigeon and on the skin and comb of a pullet. Both of these birds, within two days, showed very definite lesions which increased in degree of severity; after seven days the pigeon was killed and the virus passaged to two further pigeons and to two young cockerels. For comparison, two other young cockerels were inoculated with pigeon pox virus obtained from the Ministry of

Agriculture. The fowls inoculated with the pigeon-passaged sparrow pox developed much more severe lesions than those treated with pigeon pox; in the former, the inoculated skin follicles were greatly swollen within five days but the reaction had almost disappeared after a further six days, whereas in the latter the reaction was slower in developing, milder and slower in disappearing. Unfortunately, no fowl pox virus was available at the time but the cockerels recovered from the pigeon-passaged sparrow pox, were tested with pigeon pox virus 24 days later; no lesions developed.

II.—EXPERIMENTAL INVESTIGATION

The virus was received in the form of a suspension in glycerine-phosphate solution of crusts from sparrows showing the spontaneous disease. This was rubbed into the skin of six canaries in a region from which feathers had been removed. Three were inoculated with the first strain (20.4.33) and three with the second (1.5.33) but no differences between the lesions produced were noticed.

Except for one bird which was killed for histological material on the eighth day, all canaries died of the disease induced, death occurring 12, 13, 15, 17 and 19 days after inoculation. All showed a raised plaque-like lesion at the site of inoculation with a variable degree of involvement of the skin beyond the main lesion.

From the canary killed on the eighth day a membrane filtrate was prepared according to the technique used in studies on Kikuth's canary virus, with a permeable (0·78μ A.P.D.) membrane. This was inoculated into canaries in three successive 100-fold dilutions and undiluted on to the chorioallantoic membrane of two developing eggs. Neither of the eggs showed lesions and only the canary receiving undiluted filtrate developed the disease. This bird, which was inoculated by a combined percutaneous and subcutaneous injection, showed a much more diffuse lesion than the previous ones when it died on the 15th day after inoculation. Except that the subcutaneous exudate was smaller in amount and more cellular the lesion almost exactly resembled those found with Kikuth's canary virus and fully described previously (Burnet, 1933). There was the same thickening and opacity of the skin spreading out from the point of inoculation with swelling and cellular accumulations in the underlying subcutaneous tissue and muscle. It was clear, however, that the filtrate contained far less virus than was the case with similar preparations of Kikuth's virus. This difficulty in obtaining an active membrane filtrate has persisted. Another filtrate through a 1·0μ A.P.D. membrane was again active only when undiluted. A fresh batch of canary material, skin and subcutaneous exudate, was ground up with quartz powder and broth and divided into two parts. One was subjected to tryptic digestion overnight, the other to repeated freezing and thawing. This was followed by sand and pulp and permeable membrane filtration according to the usual technique. The filtrate from trypsinised material was inactive while that from

the frozen and thawed material infected at 1 : 100 but not at 1 : 1,000 in the canary, and on the chorioallantoic membrane of two eggs gave a few typical " pock " lesions.

This difficulty in filtration was in marked contrast to the ease with which high-titre filtrates of Kikuth's virus were obtained but the cause of the difficulty could be deduced from some microscopical observations made by Mr. J. E. Barnard on the subcutaneous exudate. This was found to contain enormous numbers of elementary virus particles in every respect similar to those of Kikuth's virus except that instead of being freely dispersed they were clumped into masses embedded in amorphous material. Relatively few solitary particles were present and it was obvious that such material would almost entirely fail to pass any membrane filter.

Once a positive lesion had been produced on the egg membrane subsequent inoculations in series were readily performed giving the massive proliferative lesion characteristic of all the bird pox strains in this situation. After three passages on the egg a typical four-day membrane lesion was ground up in 5 c.c. of broth centrifuged and the supernatant fluid diluted serially and inoculated intramuscularly in 0·25 c.c. amounts into canaries with the results tabulated :—

Undil.	+ 9 d.	
1 : 100	+ 9 d.	
1 : 1,000	+ 9 d.	+ 10 d.
1 : 10,000	+ 10 d.	+ 10 d.
1 : 100,000	+ 12 d.	+ 13 d.

All birds showed the spreading lesion characteristic of Kikuth's virus with occasionally a more massive lesion near the point of inoculation. The virus had therefore multiplied to high titre in the chorioallantoic membrane and, as judged by the shorter incubation period, had increased slightly in virulence for canaries.

In no instance throughout the experiments had any canary shown positive lesions and recovered so that, as with Kikuth's virus, no immunological work was possible. Two canaries showed generalised pock-like lesions scattered over the body and wings and quite unconnected with the spreading local lesion.

Histological sections of the canary lesions showed a general resemblance to the appearances found with Kikuth's virus but in some respects approached more the typical fowl pox picture. Bollinger-type inclusion bodies were extremely numerous, and it was not infrequent to find practically all the cells forming a feather follicle containing well-defined inclusions. In parts of some sections the general epithelial hypertrophy with contained inclusion bodies was precisely similar to that figured for Kikuth's virus in the previous paper. The subcutaneous tissue showed close packed cellular accumulations of histiocytes, many showing ill-defined acidophilic granules and vacuoles.

SUMMARY

Three cases of avian pox in wild sparrows have been described; the virus seems to resemble canary pox in causing a fatal disease in sparrows and canaries and localised lesions in fowls and pigeons.

The lesions produced in the canary by this virus were clearly intermediate in character between the acute, spreading lesion of Kikuth's virus and the localised lesion characteristic of fowl and pigeon pox. Kikuth's virus was originally detected only in the course of bird-to-bird inoculations of malarial blood but the present strain was derived from a natural disease of sparrows presenting the usual characteristics of an avian pox disease. This seems to strengthen the evidence for the view previously put forward that Kikuth's virus is a true avian pox type and not, as suggested by Kikuth and Gollub (1932) and by Bechold and Schlesinger (1933), a virus closely related to fowl plague.

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AN EXPERIMENTAL STUDY OF *TRYPANOSOMA RHODESIENSE* INFECTION IN ZEBU CATTLE

By

J. K. H. Wilde and M. H. French,

Veterinary Research Laboratory, Mpwapwa, Tanganyika Territory

INTRODUCTION

The course of *T. rhodesiense* infection in cattle has not been described in detail. Recently, there have been greatly increased cattle movements in countries where human sleeping sickness caused by *T. rhodesiense* is endemic in some areas. In studying the epidemiology of the human disease, therefore, the question whether the bovine can act as a carrier of *T. rhodesiense* has come to the fore. It was largely with the object of obtaining a definite answer to this question that the present work was undertaken.

From much detailed work by several writers, notably Corson, Duke and Fairbairn, on *T. rhodesiense* infection in the human host and in mammals other than the bovine, it has been shown that the parasite can exist in wild animal reservoirs and can be passaged cyclically through many species of such animals, as also through domestic sheep and cattle and through laboratory animals, without losing any of its virulence for man (Corson, 1935, 1936, 1937; Fairbairn, 1937). Carmichael and Mettam, in Uganda, infected cattle with the trypanosome, but their results were not conclusive. Carmichael (1933) described artificial infection of 12 cattle with *T. rhodesiense* (Tinde III strain) and nine of the animals died of typical trypanosomiasis. According to him, "cattle are more resistant to *T. rhodesiense* than they are to a known virulent strain of *T. brucei*," but "a high percentage die after a long protracted infection." Mettam (1934) said "that *T. brucei* and *T. rhodesiense* are definitely pathogenic to stock for about six months after infection, but that if the animals are cared for and well fed they steadily put on weight and recover." Carmichael (1937) commented on an experiment with *T. rhodesiense* in Ankole and Zebu cattle which was unfortunately terminated by lightning which killed all the experimental animals. He concluded, however, ". . . it would seem that *T. rhodesiense* is of low pathogenicity to both types of cattle, the Ankole type, however, tending to harbour the parasite longer than the Zebu." Hornby has stated as his opinion (private communication) that the course of *T. rhodesiense* infection in cattle is similar to that of *T. brucei*. This opinion is based on a wide experience of trypanosomiasis over many years and is backed by deductions from field observations.

The morphological similarity between *T. rhodesiense* and *T. brucei* has confused the issue in certain experiments recorded in the literature, especially where observations have been confined to field investigations. In the experiment described here, therefore, every effort has been made to obviate such complications and to follow the course of pure *T. rhodesiense* infection in the bovine animal as completely as possible and including the clinical, physiological and pathological aspects.

EXPERIMENTAL WORK

The subjects were six Zebu oxen which had been part of a herd living for several months in an area which, as far as observations by the routine methods of the Tsetse Research Department had shown, was fly-free. The herd had been under continuous observation, and representative blood samples had been taken frequently and examined for the presence of parasites, with negative results. At the commencement of the experiment the six cattle were housed and put on a maintenance ration of hay and green lucerne. The reason for this was that, should we wish to repeat the experiment or conduct parallel ones, there would be no difficulty in reproducing the same dietary conditions.

Before infection with *T. rhodesiense*, blood examination of these oxen by means of both wet and thick dry smears was made daily for seven days. The custom adopted whenever possible, as a routine, at this laboratory was followed of drawing blood from a small ear vein for examination in the early morning. This had been shown by Hornby and Bailey (1930) to be the most reliable method of demonstrating blood trypanosomes in light infections. On the day before the injection of *T. rhodesiense* into the oxen 20 c.c. of blood were drawn from each animal into citrate, and each sample was inoculated into a correspondingly numbered goat. All six goats had previously been examined daily for seven days and no trypanosomes had been found in their blood. Smears taken afterwards from these goats at weekly intervals for five months showed no trypanosomes. The six oxen on entering the experiment may therefore be taken to have been free from trypanosomes.

The trypanosomes used were *T. rhodesiense*, Kahama strain (Corson, 1936) received from the Tinde Sleeping Sickness Laboratory by the courtesy of Dr. H. Fairbairn. They had been passaged since isolation from a human case of sleeping sickness through sheep, Thompson's gazelles and cercopethecid monkeys. They were conveyed to the Mpwapwa laboratory in white rats. Blood from these rats showing a swarming infection was injected subcutaneously into the oxen. This constituted the only needle or non-cyclical passage of the trypanosomes since their isolation. The dose of blood injected was such as to ensure each ox receiving several thousands of the parasites. At the same time guinea-pigs were infected from the rats.

OBSERVATIONS

Microscopic examination

Wet and thick dry smears were examined from each ox daily until the trypanosomes appeared. Thereafter, the examination of wet smears was discontinued, but daily examination of thick dry smears was maintained throughout the experiment, no single day being missed for over six months. Trypanosomes appeared in the

peripheral blood of all six oxen. These were typical *T. rhodesiense*, mostly long forms with centrally placed nuclei with very occasionally short forms. None showed posterior nuclei. The number of days which elapsed after injection before the trypanosomes appeared in the peripheral blood varied from seven (in Ox 32) to 13 (in Ox 33), the average being 9·3. Thereafter the parasites disappeared and reappeared at spasmodic intervals for several months. At first the appearances were fairly frequent but in no case were the parasites found in slides from any one beast for longer than six consecutive days (Ox 35). Later, the intervals between appearances grew longer and trypanosomes were found in slides only on odd single days. (Charts 7 to 12.)

Prescapular Glands. Smears taken at weekly intervals never showed any trypanosomes.

Physiological examination of the blood

At intervals throughout the experiment blood samples were taken and examined.

Red and White Cell Counts

Variations in red and white cell counts occurred in all oxen but no obviously significant correlation with the course of the disease was revealed. Contrary to the effects registered by *T. congolense* disease in cattle and *T. brucei* disease in equines, no variation was shown so marked as could be described as a clinical anaemia.

The haemoglobin value varied in all, but the curves for the different oxen showed no common relationship with each other except that from the beginning to the end of the experiment there was a fall generally in the haemoglobin percentage which, however, never reached a level lower than 62. This percentage cannot be considered as a low value for this type of cattle at this time of the year, namely, the dry season.

TABLE I

NUMBER OF RED CELLS PER C.MM. OF BLOOD IN MILLIONS

Ox No.	*Day previous to injection with trypanosomes*	*Weeks after injections*					
		2	4	6	11	15	20½
		All oxen now positive for T. rhodesiense					
31	7·76	6·51	7·05	7·79	7·56	6·89	6·94
32	9·56	7·17	7·48	7·06	6·94	8·48	6·65
33	9·04	8·18	8·74	6·69	11·88*	6·85	6·89
34	9·50	8·10	8·24	9·09	8·43	9·52	7·31
35	8·67	8·96	8·51	10·31	8·43	7·45	9·01
36	9·00	9·38	8·48	9·15	10·48	7·54	7·94

* This figure is very high. It is not supported by a corresponding increase in haemoglobin value, and so may be due to an error in counting.

TABLE II

NUMBER OF WHITE CELLS PER C.MM. OF BLOOD

Ox No.	*Day previous to injection*	*Weeks after injections*					
		2	4	6	11	15	20½
32	27,700	8,700	10,000	11,800	10,000	9,000	8,000
32	21,000	8,300	8,750	13,200	9,700	12,000	16,800
33	16·700	16,500	15,000	9,400	16,500	12,000	17,000
34	9,000	19,000	10,000	13,900	11,500	14,000	11,250
35	11,100	17,000	14,400	14,900	15,300	19,000	15,000
36	19,000	8,300	11,000	17,700	16,200	20,000	15,600

Plasma Protein Changes

Determinations of the various protein fractions in the plasma of these *T. rhodesiense* infected cattle were made because a number of workers have shown that marked disturbances in the relative proportions of the different protein fractions occur during trypanosomiasis. This disturbance is not confined to the disease produced by one species of trypanosome but appears to be a general phenomenon since it has been observed with a number of trypanosome species in several different hosts.

A reduction in the albumin : globulin ratio has been reported for *T. brucei* in the dog (Mayers, 1905), *T. gambiense* in humans (Sice *et al.*, 1931), *T. berberum* in rabbits and guinea-pigs (Trensz and Jardon, 1933), *T. congolense* in cattle, sheep and a donkey (French, 1938a), and *T. brucei* in sheep, guinea-pigs and a donkey (French, 1938a). In previous work (French, 1938) from this laboratory, it has been demonstrated that the plasma protein changes produced by *T. congolense* and *T. brucei* are similar in nature irrespective of which species of domestic stock happens to be the host but that the extent of the changes varies considerably, depending upon whether a " virulent " or a " mild " strain of a trypanosome is the infective agent. The most important changes observed were an absolute and relative increase in the 47 globulin fraction up to death or crisis and a decrease in the albumins. In those animals which recovered from the disease, whether naturally or after therapeutic aid, the albumin moiety returned to normal levels in a shorter period, after crisis or treatment, than did the euglobulin fraction. It was also observed that a " virulent " type of *T. brucei* caused greater disturbances than did a " virulent " *T. congolense* strain when sheep and donkeys were the experimental animals and that, with *T. congolense* in cattle, the acute disease caused by a " virulent " strain produced greater plasma protein disturbances than were found if a " mild " strain set up the infection. In this earlier work it was also shown that, when a chronic disease was produced by the " virulent " strain of *T. congolense* in which the clinical symptoms were somewhat more marked than were found in the " mild " infections, the extent of the protein changes was also somewhat more marked. It appears, therefore, that the severity of the trypanosomiasis is roughly correlated with the extent of the plasma protein disturbances.

In the present study, and in the previous work, the plasma proteins

were fractionated with sodium sulphate according to the technique of Howe (1921), whilst the nitrogen determinations were made by the micro-Kjeldahl method. It is realised, of course, that with a single precipitation it is not possible to obtain a given protein fraction uncontaminated with other fractions just as it is appreciated that it is not yet certain whether eu- and pseudo-globulins exist as such in untreated plasma, but are more probably artifacts produced by the precipitating conditions. On the other hand, by standardising the details of separation and particularly the electrolyte concentration, fractions can be split off from the plasma proteins which are capable of being reproduced and which are in rough agreement between different workers. In fact such fractionation has been of great value in the concentration of antitoxic sera. These fractions have been determined in this investigation because there is no more convenient method available at Mpwapwa for following plasma protein alterations and they can serve a very useful purpose in view of the present uncertainty concerning the nature of the plasma globulins and albumins. The following comments on eu- and pseudo-globulin and albumin are made subject to the above qualifying remarks.

Total Protein. The changes in the total protein content of the plasma during *T. rhodesiense* infection of cattle are not marked nor are the nature of the variations constant.

In this respect the observed changes agree with those recorded in other trypanosomiases such as *T. gambiense* in humans (Sice *et al.*, 1931), *T. evansi* in rats (Krijgshman, 1934), *T. annamense* in rabbits (Launoy and Lagodsky, 1937), *T. congolense* in cattle, sheep and a donkey (French, 1938a), and *T. brucei* in sheep, guinea-pigs and donkeys (French, 1938a).

Non-Protein Nitrogen (*N.P.N.*). The N.P.N. showed irregular small variations but there is no constancy about the nature of the changes in the different animals.

The finding is also in conformity with the observations on other trypanosomiases such as *T. equiperdum* in rats (Linton, 1930), *T. evansi* in horses (Randall, 1934), *T. annamense* in rabbits (Launoy and Lagodsky, 1937), *T. congolense* in cattle, sheep and a donkey and *T. brucei* in sheep, guinea-pigs and donkeys (French, 1938), although in some of this previous work increases have been found in the N.P.N. fraction at crisis or in the pre-mortal stages.

It is probable that no appreciable kidney damage occurred in the comparatively benign reactions following *T. rhodesiense* injections in the present investigations and consequently there would be no increase in N.P.N., and particularly in the urea fraction.

Fibrinogen. No appreciable change was found in the proportions of fibrinogen in this study, and this agrees with the previously reported changes in *T. congolense* and *T. brucei* infections (French, 1938a).

Pseudoglobulin. Only small and irregular variations were observed in the pseudoglobulin proportions, a fact which agrees with the findings recorded with *T. congolense* and *T. brucei* (French, 1938).

Euglobulin. The absolute and proportional amounts of euglobulin increased quickly soon after injection of *T. rhodesiense* into these cattle. The euglobulin proportions reached their maximum levels somewhere between the sixth and 15th weeks after injection of the trypanosomes but the point of maximum euglobulin content varied in the different cases. Afterwards there was a steady decrease towards normal proportions from the maximum value but in no case had the amount of euglobulin returned to normality $20\frac{1}{2}$ weeks after injection.

The euglobulin changes were therefore similar in type to those reported for *T. berberum* in rabbits and guinea-pigs (Trensz and Jardon, 1933), *T. congolense* in cattle, sheep and a donkey and *T. brucei* in sheep, guinea-pigs and donkeys (French, 1938a). An increase in the proportions of euglobulin have also been reported from *T. soudanense* in camels (Horgan and Bennett, 1929) but, unlike all the other records, this increase was found to take place at the expense of the pseudoglobulin and not the albumin fraction.

Albumin. The absolute and relative amounts of albumin in the plasma proteins fell fairly quickly after infection was established and the minimum proportional values were recorded at the time of maximum euglobulin content. With the one exception noted above, this finding is in agreement with other observations on trypanosomiases.

Since the euglobulin increase was practically entirely at the expense of the albumin fraction it appears that infection with *T. rhodesiense* either (*a*) caused an increase in the dimensional proportions of the plasma protein molecules or (*b*) altered the conditions of the plasma or its proteins so that larger proportions of its proteins were precipitated at lower electrolyte concentrations.

It is interesting to observe that in these very mild cases of trypanosomiasis, where the degree of anaemia, the thermal reactions, the liveweight changes and the clinical symptoms were slight and less marked than those recorded for certain mild cases of *T. congolense* in cattle (French and Hornby, 1934), the disturbance in the euglobulin levels was, with the exception of Ox 35, somewhat less than was found in the mild *T. congolense* infections. Taken in conjunction with the previous records, the present results support the view that in trypanosomiases the degree of plasma protein disturbance is correlated with the severity of the disease.

Owing to lack of data on the changes produced in cattle plasma proteins by *T. brucei*, it was unfortunately not possible to compare the *T. rhodesiense* changes directly with those caused by the morphologically similar *T. brucei*. Such a direct comparison would be of great value in discussions on the relationships of these two trypanosomes. On the other hand, the present results have demonstrated that, when *T. rhodesiense* produced but a very mild disease in bovines, the disturbances in the plasma protein fractions were of exactly the same type as, but smaller in extent than, those produced by other pathogenic trypanosomes.

Blood Sugar Changes

Much has appeared in the literature concerning the changes in the blood sugar levels during the various trypanosomiases, but since this work was recently reviewed from this laboratory (French, 1938–42) it is not proposed to repeat the summary of the previous findings except to say that in fatal infections of small animals and in some infections of larger animals a hypoglycaemia develops in the pre-mortal stages. It was found in previous work (French, 1938b), that there was no gradual development of hypoglycaemia during *T. congolense* and *T. brucei* infections of large animals but that a sharp fall in the blood sugar level occurred shortly before death or crisis. In animals that were treated successfully or recovered without therapeutic aid, the blood sugar level returned to normal immediately the crisis was passed.

In order to see if a similar state of affairs occurred during *T. rhodesiense* infections of cattle, blood sugar determinations were started in the six animals under experimentation. Unfortunately it was not possible to make blood sugar estimations every time the plasma protein fractions were determined but the following table summarises the results obtained. The blood sugar was determined by the method of Hagedon and Jensen (1918).

BLOOD SUGAR VALUES (MGM./100 ML.)

Weeks after infection	0	2	4	6	11	15	20½
Ox 31	69·5	67·3	—	69·9	66·7	—	—
Ox 32	67·6	66·7	—	69·3	67·2	—	—
Ox 33	70·6	68·5	—	68·1	69·3	—	—
Ox 34	65·8	69·3	—	67·6	64·7	—	—
Ox 35	68·3	65·8	—	70·6	68·1	—	—
Ox 36	64·7	70·5	—	68·5	65·8	—	—

It is regretted that the data are incomplete but, in every case the maximum euglobulin content was reached before the eleventh week after infecting with parasites. The blood sugar level in oxen varies rather erratically, yet the above figures are well within the normal range and there is no suggestion of hypoglycaemia and certainly no progressive hypoglycaemic tendency as a result of *T. rhodesiense* infection.

Sedimentation Tests

The effect of various pathological conditions on the rate of sedimentation of red blood corpuscles in citrated or oxalated blood has been the subject of much research in the past and from this volume of work have emerged the facts that increases in the fibrinogen or euglobulin and decrease in the albumin contents of the plasma permit a more rapid sedimentation of the red cells. The exact

relationships of the plasma changes to the sedimentation rates are not known.

Since previous work (French, 1937) had shown that, during the course of *T. brucei* and *T. congolense* infections in domestic livestock, red cell sedimentation rates were increased it was decided to follow the rates during the present work on *T. rhodesiense* in the hope that any variations would act as confirmatory evidence for any disturbances found in the plasma protein fractions. Unfortunately it was not possible to make a complete record of the sedimentation rates but the figures in the following table summarise the results obtained. In each case the figure given represents the mm. of clear plasma resting over the sedimented red cells when 1 ml. of citrated blood was allowed to stand for 1 hour in a capillary tube of such dimensions that the blood occupied 265 mm.

RATES OF RED CELL SEDIMENTATION

Weeks after infection	0	2	4	6	11	15	20½
Ox 31	1·0	1·0	—	1·1	1·3	—	—
Ox 32	0·9	1·1	—	1·3	1·1	—	—
Ox 33	1·0	0·9	—	1·2	1·1	—	—
Ox 34	0·9	1·1	—	1·4	1·3	—	—
Ox 35	0·8	1·3	—	1·6	1·6	—	—
Ox 36	0·9	1·4	—	1·2	1·3	—	—

It is seen that very little effect on the rate of red cell sedimentation was observed though in all cases there is a very small increased sedimentation.

CLINICAL EXAMINATION

Throughout the experiment the rectal temperatures of the experimental oxen were recorded daily in the early morning. In the early stages, a certain fluctuation of temperature was noted in all the animals. This may have been influenced by the fact that the beasts had not yet accommodated themselves to the process of temperature recording and blood examination, but it is felt that the temperature changes can be attributed only in part to this cause, and that this erratic thermal variation was to some extent due to the trypanosome infection, especially as some of the more prominent peaks coincided with the appearance of trypanosomes in the peripheral circulation. However, there was no manifestation of a regular, definite thermal reaction. (Charts 7 to 12.)

Occasionally throughout the experiment, some of the animals showed a slight inappetence or a mild malaise for a very short period. This did not appear to bear any constant relation to the appearance of the parasites in the blood and at no time did any of the infected cattle give the impression of being " fly-struck."

The oxen were weighed weekly and did not lose weight in any significant manner, but maintained a level deemed compatible with the ration received. After 4½ months from the commencement of the experiment, this ration was increased by the addition of a few pounds of concentrates per beast (cotton seed, rice polishings, oil cake and mineral mixture) and the animals were allowed out to graze. A slight, but not well-marked improvement in condition was noted subsequently (Charts 7 to 12. Note rise in weight curve commencing after the 18th week).

PATHOLOGY

Six months after inoculation blood was drawn from all the oxen and was injected directly into corresponding goats on two successive days (20 c.c. per day). The goats had been under close observation by blood slide examination for several months and had never shown trypanosomes in that period. The blood was drawn and injected on two successive days in order to reduce the possibility of the trypanosomes, if present, escaping detection. Two of the beasts, Nos. 35 and 36, which had shown the presence of trypanosomes in the blood on more occasions than the others, were then slaughtered for *post-mortem* and histopathological examination. The findings are given below :—

Ox No. 35

Condition, good ; external appearances, normal.

All organs were examined and no abnormalities were noted except a little excess of white lymphatic substance in a pharyngeal haemolymph node. Slight congestion in a few small patches on the surface of the cortex of both kidneys. The periportal lymph gland was slightly haemorrhagic. The liver was a little indurated and contained a few flukes (*Fasciola gigantica*). (These were probably responsible for the induration.) The sub-lumbar glands were slightly haemorrhagic. There were signs of an old localised pleurisy with small adhesions (resolved). One small area of lung was congested. The brain showed the presence of blood clots, caused undoubtedly by stunning. There was slight erosion of the mucous membrane at the junction of the caecum and colon.

All these details were of a very minor nature, and there was no suggestion of the debilitated appearance so common in carcases infected with *T. congolense*. The carcase of ox No. 35 would have been passed as " prime " in a public abattoir. Nevertheless, portions of the following tissues were taken for histopathological examination and for the careful examination of sections (especially stained with Geimsa) for the presence of trypanosomes.

Parotid gland, pharyngeal haemolymph node, dorsum of tongue, popliteal lymph gland, pancreas, spleen, kidney, liver, gall bladder wall, hepatic lymph gland, neck of urinary bladder, sub-lumbar gland, mesenteric haemolymph node, subthoracic haemolymph node, wall of right ventricle, congested portion of lung, cerebellum, cerebrum, gastric lymph gland, eroded area at junction of caecum and colon.

Sections of these tissues stained with haematoxylin and eosin were examined carefully without revealing any abnormalities other than those

expected in the kidney, lung and ileo-caecum from macroscopic examination. Those sections stained with Geimsa also failed to show the presence of trypanosomes or other abnormalities excepting those noted above. (The portion of lung chosen showed broncho-pneumonia, but this was due to the presence of parasitic worms which were demonstrable in the sections).

Ox No. 36

Condition, fair to good ; external appearances, normal.

All the organs were examined and no abnormalities were noted except as follows : The gall bladder showed marked pathological changes. The contents were of a pale greenish-yellow colour having a purulent appearance and of semi-solid consistency. The mucous membrane of the viscus was heavily inflamed and black in colour with large areas of ulceration of the mucous membrane in a ring round the neck of the organ. A small area of the liver adjacent to the neck of the gall bladder was softened and showed early necrotic changes. Otherwise the liver was quite healthy in appearance. Smears were taken from the inspissated contents of the bladder, but these did not show the presence of any organisms.

The carcase of this ox, although not in as good condition as that of No. 35, was provided with a fair amount of fat and would have been passed as healthy in a public abattoir. It compared very favourably with much of the slaughter stock at this time of the year (the end of the dry season.) In spite of this, however, portions of the following tissues were taken for histo-pathological examination and for examination for the presence of trypanosomes.

Kidney, adrenal gland, periportal lymph gland, normal liver tissue, tissue from the softened portion of the liver near the gall bladder, spleen, cerebrum, pancreas, ulcerated gall bladder wall, neck of gall bladder (ulcerated and non-ulcerated areas), Peyer's patch, mesenteric haemolymph node, diaphragmatic muscle, gastrocnemius muscle, pulmonary haemolymph node, lung, blood colt from right ventricle, left auricular-ventricular valve, myocardium of the right ventricle, ileocaecal valve, bone marrow from the humerus. With the exception of the gall-bladder sections, none of these tissues showed abnormalities or trypanosomes. The sections of gall-bladder tissue, as would be expected, showed heavy congestion, large numbers of granular cells, and erosion of the mucous membrane and lamina-propria.

It is our view that the cholecystitis and its concurrent manifestations are unconnected with the trypanosomiasis although the possibility of connection cannot be ruled out entirely.

In addition guinea-pigs were injected with cerebrospinal fluid and aqueous humour from ox No. 36 without revealing the presence of trypanosomes.

The goats which received the blood from the six oxen, prior to the slaughter of Nos. 35 and 36, were examined daily by means of blood slides. Three of them, *viz.*, those receiving blood from oxen Nos. 33, 34, and 35 showed the presence of *T. rhodesiense* in their blood after 28–29, 19–20 and 8–9 days respectively. It is therefore apparent that these three oxen were harbouring the trypanosome in their bodies and it is reasonable to assume that the others, *viz.*, Nos. 31, 32 and 36, were not carrying the trypanosome in their blood stream. Thus, the parasite had been either entirely thrown off,

TABLE III

HISTORIES OF GOATS USED IN *T. rhodesiense* TESTS

Blood examined at weekly intervals for a period of 24 weeks. No trypanosomes seen

Received 20 c.c. of blood from Oxen Nos. 31, 32, 33, 34, 35 and 36 respectively—*October 2nd*
Received 20 c.c. of blood from Oxen Nos. 31, 32, 33, 34, 35 and 36 respectively—*October 3rd*

Daily Blood Slide Examinations

October																															*November*												Goat No.
4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12				
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—				1
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—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+ ve L	+ ve L	+ ve L	—	—	—	—	+ ve L	+ ve L	—	—	—	+ ve L	+ ve L	—	—	—	—	—	—	—	—				4
—	—	—	—	—	—	+ ve L	—	—	—	—	—	—	+ ve L	—	—	—	—	+ ve L	+ ve L	+ ve L	+ ve L	—	—	—	—	+ ve L	—	—	—	—	+ ve L	—	—	—	—	—	—	—	—				5
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—				6

— = No trypanosomes seen. + ve = Trypanosomes seen. L = Long forms.

or may have been retained in some organ or organs of the body. The fact that no trypanosomes were found in the histological examination of the tissue of oxen Nos. 35 and 36, does not preclude the possibility of their presence in these or other tissues, as the demonstration of the parasites, even in those animals regularly showing positive blood slides, is extremely difficult. As a check on the method of sectioning and staining for the presence of trypanosomes, organs from a rat, showing a heavy infection of *T. rhodesiense*, were fixed, embedded, sectioned, and stained by means of the same technique. Trypanosomes were found in these sections with difficulty and those seen were located in small blood vessels.

DISCUSSION

We are led by the observations made in this experiment to the conclusion that *T. rhodesiense* is only mildly pathogenic for Zebu cattle. The trypanosome is capable of maintaining itself and multiplying in the body of the ox for a limited period which may be in the region of six months. This agrees with Mettam's statement (*vide supra*) as does also the fact that with care and good feeding the oxen appear to be able to subdue or even throw off the trypanosome after that period has elapsed. In the early stages of infection, the parasite does evoke a reaction in the bovine host, but this is of a very mild and indefinite nature. This reaction when compared with that described by Curson (1928) for *T. brucei* in the ox is found to be milder than the latter. In our experiment, although daily blood examinations were made, trypanosomes were only found on odd days at apparently erratic intervals. But it is almost certain that blood injections of reasonable volume into goats would have proved positive for the parasite at least up to the time of its last appearance in blood slides.

If Curson (1928) is referring to this type of injection and not to blood slide examination, when he says that the trypanosome (*T. brucei*) can be obtained from the blood easily at any time in the first nine months, the two infections are similar in this respect, except that we consider that three of our oxen have thrown off the parasite in six months. This may be an indication of a milder type of infection.

Unfortunately, as is stated above in the section on plasma protein changes, information on the course of *T. brucei* disease in oxen is not available in the same detail as that given in this paper for *T. rhodesiense* infection and so no accurate comparison can be made between the two disease courses. However, from general observations on *T. brucei* disease, compared with our experience with *T. rhodesiense*, we are bound to admit a very close resemblance between the two infections and we have found nothing in our investigation to invalidate the theory that *T. rhodesiense* may be a form of *T. brucei* which has become adapted to life in the human host and has, in a

certain degree, lost the capacity to cause pathological changes in the bovine (Duke, 1935). Thus our findings are in accord with the belief of this author.

One fact which emerges very definitely from this work is that for a certain period, which may be as long as six months, the ox can carry *T. rhodesiense* in its body in such a way that it appears in the peripheral blood from time to time in such concentration as to be demonstrable in blood-slides.

Duke (1935) has shown that *T. rhodesiense*, when passaged through bush-buck, a fowl, three cattle, and two monkeys remains infective for man, and Corson's work on the passage of this trypanosome through other domestic and wild animals and back to man supports this view. (Corson, 1935 and 1936).

It is therefore reasonable to assume that during at least the first six months of *T. rhodesiense* infection, cattle can act as true carriers of the disease and so are a source of danger to humans when moving from areas where sleeping sickness is endemic to other tsetse fly-infested areas from which *T. rhodesiense* is absent.

SUMMARY

Six Zebu oxen were infected with *T. rhodesiense* (Kahama strain), and all of them supported the trypanosome.

Careful examinations were made on the blood plasma and cellular constituents and also clinical observations were maintained throughout the experiment and little physiological disturbance was noted.

The blood of three of the oxen contained the parasite for at least six months, whereas it appeared that the other three had thrown off the infection.

Post-mortem examination and histopathological examination revealed no pathological changes of any significance.

It was considered that the course of the *T. rhodesiense* infection in Zebu oxen was similar to, if somewhat milder than, that due to infection with *T. brucei*.

Discussion of the observations made led to the conclusion that oxen can act as carriers of *T. rhodesiense* for at least short periods.

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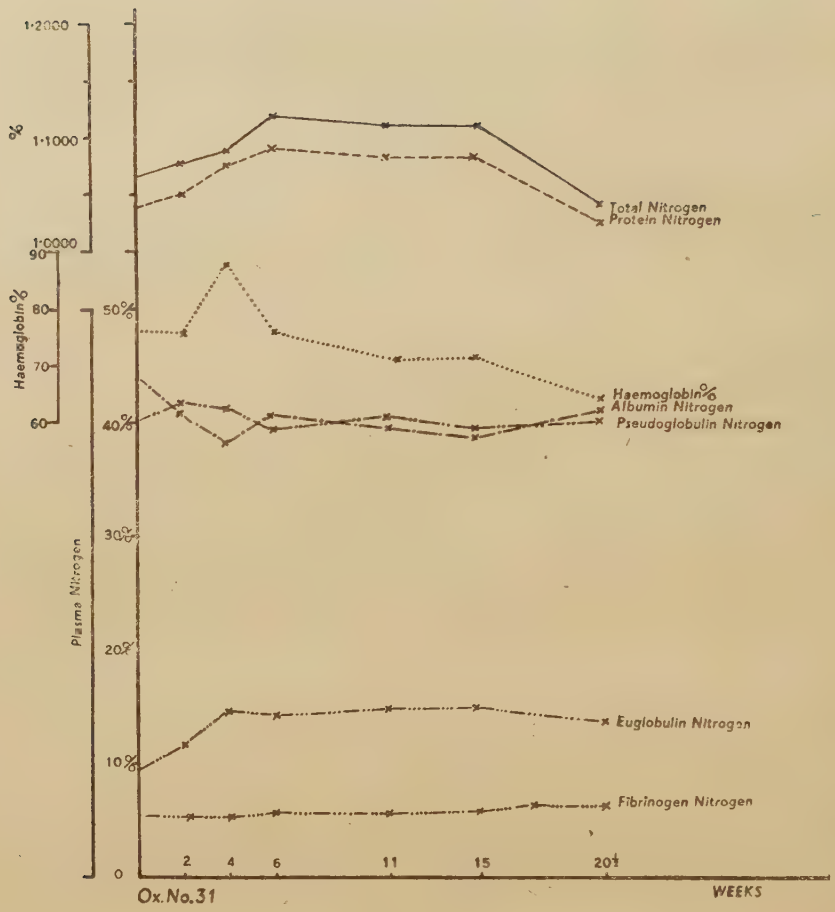
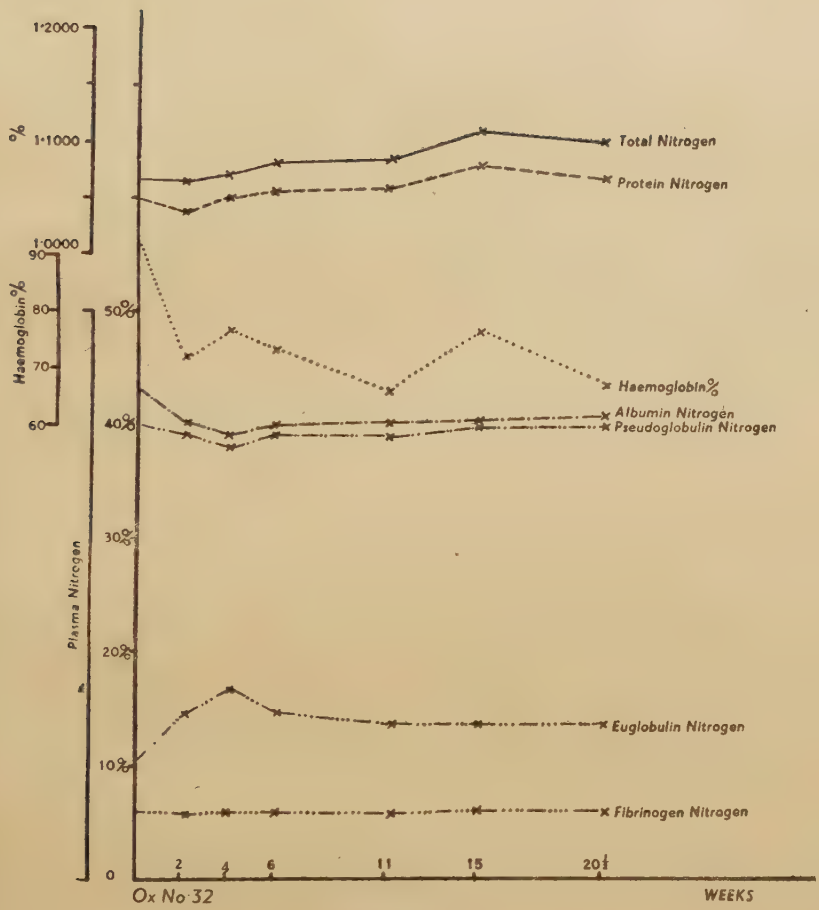


CHART No. 1

Tables corresponding to Charts 1 to 6 inclusive are available in the records of the Veterinary Research Laboratory, Mpwapwa.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1·0000
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Haemoglobin %
50%
40%
30%
20%
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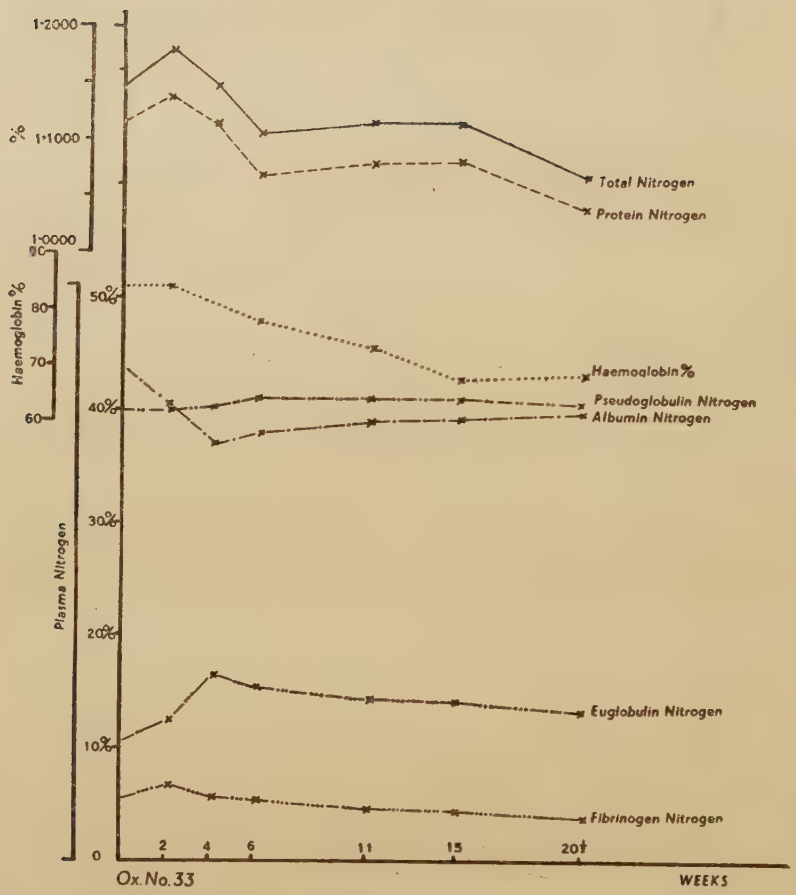
Plasma Nitrogen
Total Nitrogen
Protein Nitrogen
Haemoglobin%
Albumin Nitrogen
Pseudoglobulin Nitrogen
Euglobulin Nitrogen
Fibrinogen Nitrogen
2
4
6
11
15
20
Ox No 32
WEEKS

CHART No. 2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Protein Nitrogen
Haemoglobin %
Pseudoglobulin Nitrogen
Albumin Nitrogen
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Fibrinogen Nitrogen
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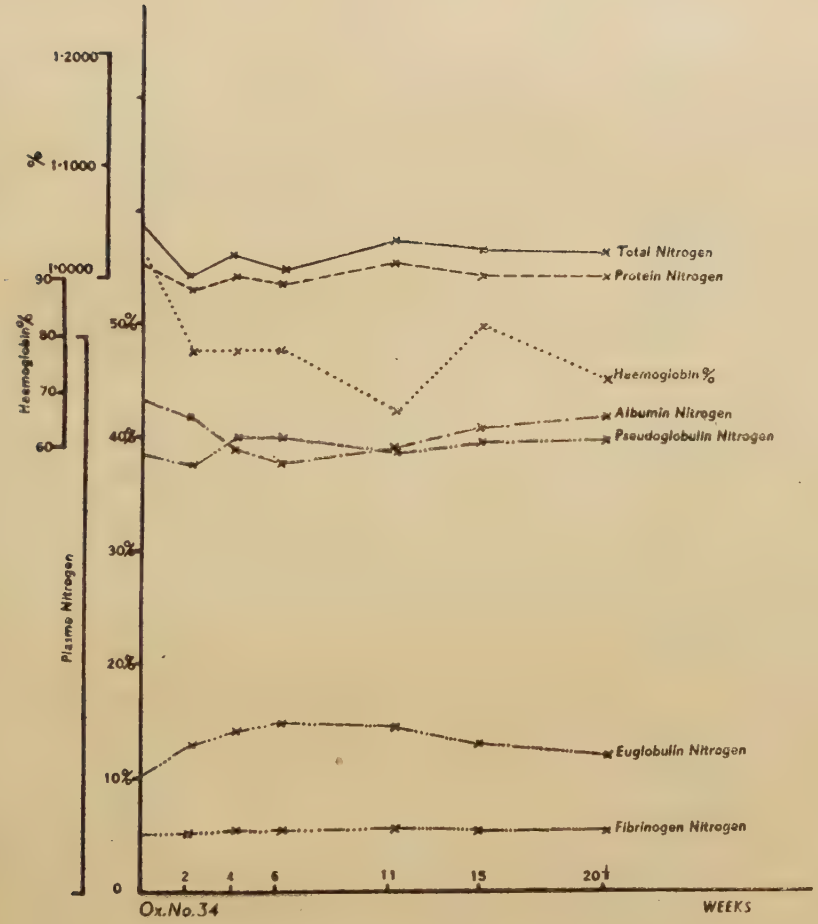
Ox. No. 33
WEEKS

CHART No. 3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Albumin Nitrogen
Pseudoglobulin Nitrogen
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Ox.No.34
WEEKS

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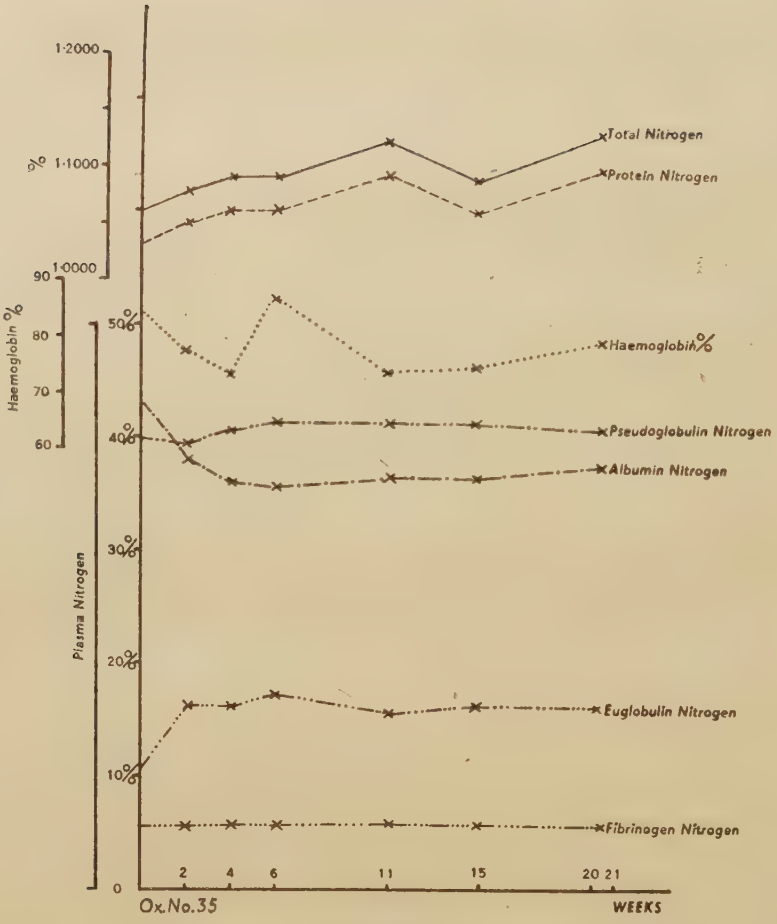
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Protein Nitrogen
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Pseudoglobulin Nitrogen
Albumin Nitrogen
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Fibrinogen Nitrogen
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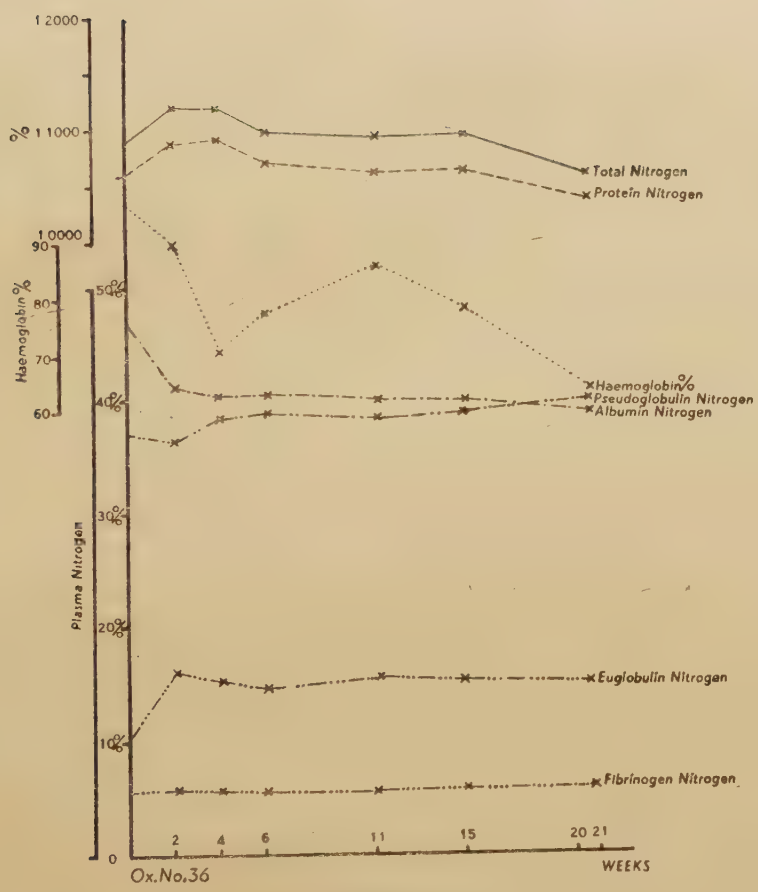
20 21
Ox. No. 35
WEEKS

CHART No. 5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Pseudoglobulin Nitrogen
Albumin Nitrogen
Euglobulin Nitrogen
Fibrinogen Nitrogen
2
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WEEKS
Ox. No. 36

CHART No. 6

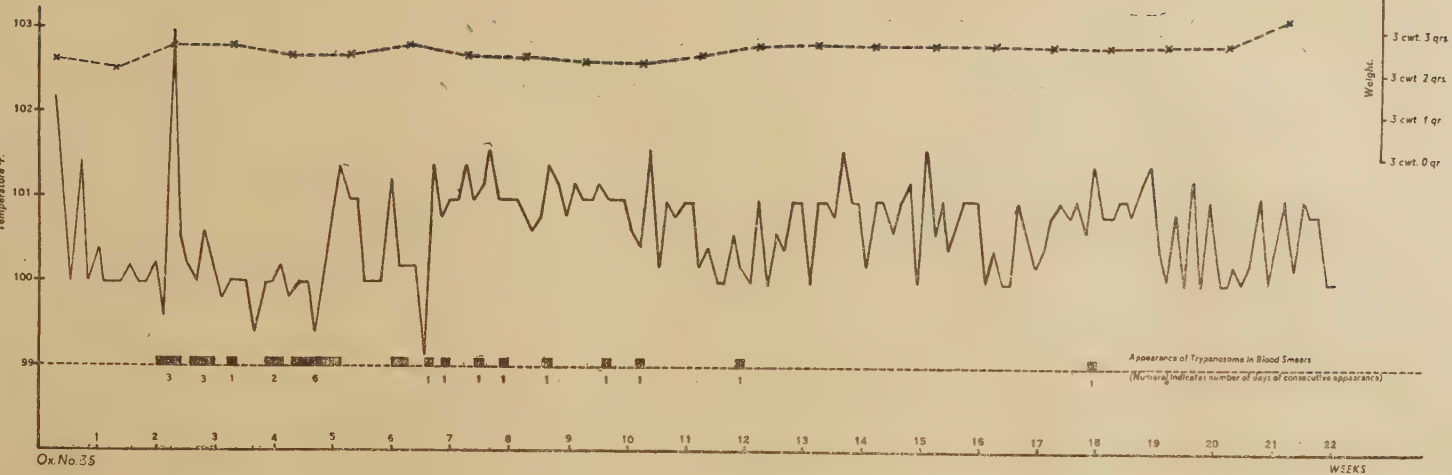
CHART No. 7

CHART No. 8

CHART No. 9

CHART No. 10

CHART No. 11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99
Weight.
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3 cwt. 2 qrs.
3 cwt. 1 qr.
3 cwt. 0 qr.
Appearance of Trypanosome in Blood Smears
(Numeral indicates number of days of consecutive appearance)
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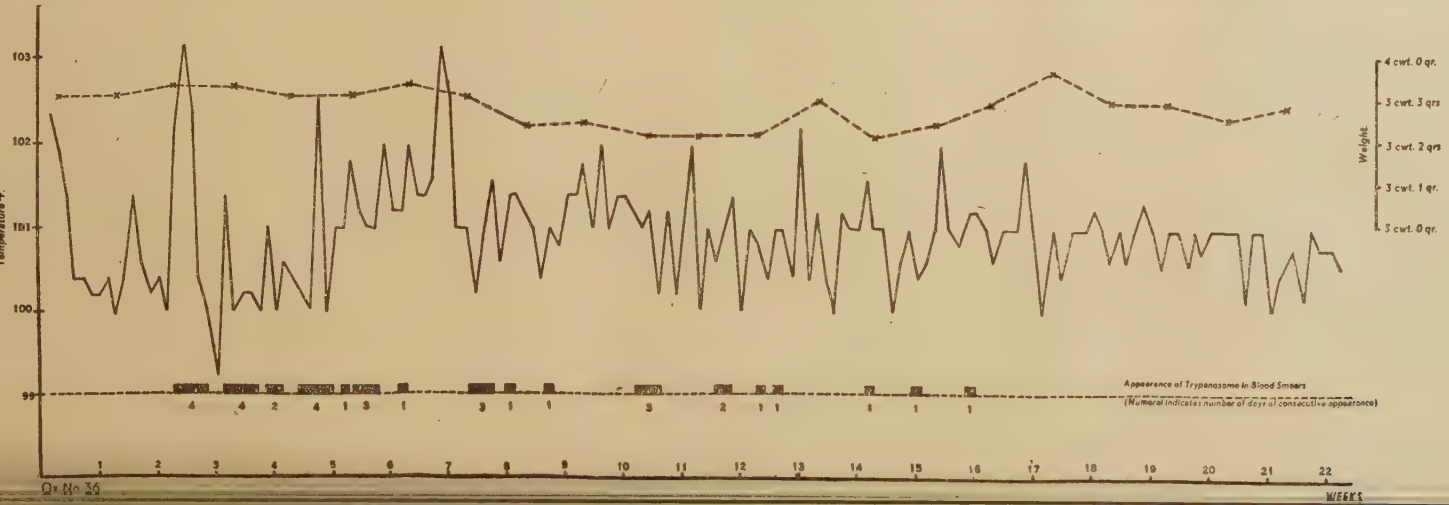
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
WEEKS
Ox. No. 35

CHART No. 12


Temperature °F.
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Weight.
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3 cwt. 0 qr.
Appearance of Trypanosome in Blood Smears
(Numeral indicates number of days of consecutive appearance)
4 4 2 4 1 3 1 3 1 1 3 2 1 1 1 1 1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
WEEKS
Ox No 36

STUDIES ON THE HAEMATOLOGY OF SHEEP

V.—A SURVEY OF BLOOD PICTURES IN SHEEP DISEASES

By

H. H. Holman

Animal Diseases Research Association, Moredun Institute, Gilmerton, Midlothian

INTRODUCTION

In previous articles (Holman, 1944a, 1944b, 1944c, 1945) standards and definitions for blood abnormalities were determined, and it now remains to apply them to sheep diseases of which the blood picture has been observed. For the classification of diseases the arrangement given in the Report on Diseases of Farm Livestock, Section 2 (Anon, 1944) has been followed.

DISEASES CAUSED BY BACTERIA

Entero-Toxaemic Diseases of Sheep

In this group death is due to the toxins of the *Cl. welchii* group of organisms. The effect of intravenous injection of fatal doses of these toxins has been investigated (Gordon, Stewart, Holman, and Taylor, 1940; Holman, unpublished), and the principal alterations in the blood picture are shown in the table. With Type A, the table shows the temporary agranulocytosis produced, lymphocytes and monocytes representing 97 or 98 per cent. of the leucocytes, and the haemolytic anaemia, indicated by low red counts accompanied by an increase in fragility. This anaemia with increased fragility also occurs in non-fatal cases. One case shows a very abnormal coagulation time. With Type B there was a leucopenia affecting both granular and non-granular cells. With Type C there were no distinct changes. With Type D there was a leucopenia as with Type B as well as haemo-concentration and some decrease in coagulation time.

Lamb Dysentery. Hare and Glynn (1927) recorded the blood picture in an outbreak of lamb dysentery. Changes were limited to leucocytosis, although most lambs exhibited a neutrophilia some hours before death. The lower average red count apparent a few hours before death was mainly due to one lamb with a count of only 5·3 mil. Fraser (1930) recorded another outbreak in which red and white counts were made on only three lambs, the picture being similar to that mentioned above for Type B toxin, that is, a leucopenia without the haemo-concentration observed with Type D.

Pulpy Kidney Disease.—Death occurs so rapidly in this disease that it is hard to procure affected sheep before death. In the case recorded in the table the blood picture resembled that for Type D toxin.

Staphylococcal Infections

Different degrees of leucocytic reaction following experimental infections with *Staph. aureus* have been described (Holman, 1944c). Cases of natural infection are recorded in the table.

Gangrenous Mastitis of Ewes. Four cases are recorded; in the second case the severity of the infection caused a leucopenia and it was sufficiently prolonged to set up toxic changes in the neutrophils.

Tick Pyaemia. The usual neutrophilic reaction is observed, and in one case a tendency to haemo-concentration is apparent.

Joint-Ill. Four cases are recorded. A pure growth of *Staphylococcus* was recovered from the synovial fluid in the two cases tested, but no neutrophilic reaction was observed presumably owing to the lesions being strictly localised.

Other Bacterial Diseases

Paratuberculosis (*Johne's Disease*). Ten cases in a late stage of infection are given. All showed a neutrophilia characterising the invasion phase of infection, although the degree of reaction varied from case to case. The erythrocytic picture for all sheep gave figures for the M.C.H.C. below the normal mean and in two the figures indicated a slight hypochromic anaemia.

Pyobacillosis (*Infection due to Actinobacillus lignieresi*). In three sheep examined from one outbreak, showing involvement of the lymph glands, abnormality, where present, was limited to a neutrophilia; the erythrocytic picture was normal although the red counts were low compared with those of three healthy sheep in the same flock. In another outbreak the haemograms were completely normal in two sheep in which the lesion was confined to an open abscess of the jaw.

Fusiformis necrophorus Infection in Lambs. Two lambs about six months old, were examined, both dying emaciated, with extensive necrotic areas of the liver at autopsy. In the first lamb, a neutrophilic reaction commenced 26 days before death, leading to a constant mild relative neutrophilia with occasional periods of leucocytosis. In the second, a slight relative neutrophilia was observed ten days before death, and later occasional periods of leucocytosis were apparent with a varying shift to the left. In both cases the neutrophilic reaction decreased with the onset of a myeloid hypoplasia before death. Oligocythaemia, apparent 23 days before death in one lamb and 33 days before death in the other, progressed to an aplastic anaemia. In a third case recorded, limited to a single sample from a three-week-old lamb which was suffering also from tick-borne fever, the change was limited to a neutrophilic reaction.

DISEASES CAUSED BY VIRUSES AND RICKETTSIA

Louping-Ill (*Trembling*). Selected figures from the blood picture in louping-ill investigated by Holman (Taylor, Holman and Gordon, 1941)

are recorded in the table. The first sample is pre-experimental. The second sample, taken two days after inoculation, shows a very slight neutrophilic reaction. The third sample, taken half-way through the course of the disease, is unremarkable, and the final sample taken 20 days after inoculation shows the eosinophilia of recovery. There is no neutropenia and staphylococcal infection can stimulate neutrophilia.

Contagious Pustular Dermatitis. Four sheep infected by scarification were examined over a period of 14 days. Scarification was followed by a slight, transient neutrophilia; later there was some decrease in eosinophils, followed on the 14th day by the eosinophilia of recovery.

Scrapie. Bleedings were carried out on 15 affected sheep. The first line in the table shows the averages for the first sample taken from each of the 15 sheep. The second line shows the averages for the last samples, taken from ten sheep, where these were taken more than nine days before death. The third line shows the last samples taken, from the five other sheep, less than ten days before death. Then alone abnormalities became apparent, consisting of eosinopenia and neutrophilia.

Tick-borne fever. The blood picture as recorded by Taylor *et al.* comprised a neutropenia followed by an eosinophilia of recovery. Haemograms from these authors' records giving the minimum neutrophil proportion observed in four lambs are reproduced in the table. In one case the figure is of very low normal value. This neutropenia is obliterated if intercurrent infection stimulates a neutrophilic reaction. Rickettsial bodies may be seen in neutrophils, eosinophils and monocytes during the febrile stage of the disease, before the onset of neutropenia.

DISEASES CAUSED BY ENDO-PARASITES

Figures for sheep suffering from a severe mixed infestation in which *Haemonchus contortus* was predominant, are given in the table. In flock A, ten sheep were sampled; three exhibited a macrocytic anaemia, three a hypochromic anaemia, and one a slight neutrophilia; in the remainder of the flock, the haemograms were normal. Changes in this flock following the administration of phenothiazine have been recorded by Holman and Pattison (1941). Among sheep sent in for diagnosis the same abnormalities can be seen, except that being more emaciated than the sheep in flock A, neutrophilia was always present, while one case showed anhydraemia due, perhaps, either to persistent scouring or to the onset of collapse.

These findings agree with those of Fourie (1931), who produced experimental infestations with *H. contortus* alone. In some cases he recorded a macrocytic anaemia and a neutrophilia that became evident some weeks after infestation. Fourie believed that macrocytosis was an attribute of post-haemorrhagic anaemia. Andrews (1942), also working with infestations of *H. contortus*, agreed that the anaemia was post-haemorrhagic in type, but did not record macrocytosis. Holman and Pattison (1941), however, suggested that where macrocytosis was also apparent, a dysfunction involving a haemopoietic principle might be involved.

In mixed infestations Fraser and later Holman and Pattison (1942) found that in the mild ones there was no alteration of the blood picture. Further, in 63 sheep where the worm egg counts varied from 0 to 2,500, no correlation could be found between the counts and the figures for haemoglobin, neutrophils or eosinophils (Holman, unpublished). On the other hand, from the figures given by Stewart and Piercy (1935) for 30 sheep in a border pine area, in which worm egg counts varied over the same range, it was seen that both the haemoglobin concentration and the eosinophil percentage were very significantly higher where the worm egg counts were under 200 per g. but that the neutrophil percentage again showed no correlation.

In heavy infestations Fraser, and, later, Stewart and Piercy (1934) reported a lower neutrophil percentage. Considering the latitude of the normal range, the lack of correlation between the neutrophil percentage and the worm egg count in 93 sheep as recorded above, and in the case of Stewart and Piercy, the fact that the highest worm egg counts (those over 8,000 per g.) correlated with neutrophilia, their statement is not convincing.

When the blood picture of those infestations in which *H. contortus* is known to be absent, or only present in sparse numbers, is inspected in the table it will be seen that there is little chance of differentiating between infestation with one species and with another by means of the blood picture. Severe infestations of various types lead to hypochromic anaemia, neutrophilia and eosinopenia. A possible exception may occur in the case of liver fluke infestation, where dysfunction of the liver may lead to an aplastic anaemia as occurs when it is infected with *F. necrophorus*.

Three cases of sturdy (coenurus cerebralis infection) have been examined (not included in table). In one the cyst was present in the lumbar region causing paralysis in a hind limb and in the other two large cysts were present in the cerebrum. None of these cases showed any tendency to a neutrophilia or a neutrophilic shift, and the blood picture was completely normal with an eosinophil percentage varying from 2 to 8, and basophil percentages of 0, 2 and 3. Haemograms from two sheep infested with coccidia gave a pattern similar to worm infestation with one sheep anaemic and the other showing anhydraemia and neutrophilia. Eosinophil percentages were normal.

A review of the literature adds little of value for differential diagnosis among the various helminth infestations. Fourie (1937) produced a pure experimental infestation with *Os. columbianum*. His records showed periods of neutrophilia nine weeks after infestation and a recurrence before death. Eosinophils increased between the fifth and eighth weeks but then receded until death ; there was a slight but continuous fall in erythrocytes to a figure of 8·4 mil. before death. Andrews (1938) produced an experimental infestation with *C. curticei* but only observed a simple leucocytosis. Wirth (1931), quoting work on liver fluke infestation, records a transient increase in leucocytes followed, in cases of severe infestation, by neutrophilia. Eosinophils increased early in infestation and could reach 47 per cent. of the count, but their proportion did not necessarily correlate with the severity of infestation. Anaemia corresponded to the degree of emaciation present.

With regard to parasitic bronchitis, Mönnig (1941) states only that anaemia may be marked, while, with regard to tape worms, Burnett (1917) names several species that produce anaemia in cows and sheep but gives no figures.

DISEASE DUE TO METABOLIC DISORDERS

Pine. The blood pictures for cobalt-deficiency pine and for " Solway " pine, including both the biochemical and cytological findings, were recorded by Stewart and Holman (1944); the averaged figures for the haemogram are repeated in the table. Normals from the same flocks have not been included. Where differences were found to occur among the affected lambs they showed a bias towards oligocythaemia, hypochromia and relative neutrophilia. These abnormalities could be explained on the grounds alone that the lambs which were emaciated were more subject to severe worm infestation. The same authors (unpublished data) fed a cobalt salt to a cobalt deficient ewe but failed to increase the red count, a result similar to that obtained by Josland (1937).

The blood picture for " Northumberland " pine, another condition dependent on a combination of deficiency and worm infestation, has been recorded by Stewart and Piercy (1935), who similarly concluded that changes in the blood picture, when present, were due to worm infestation.

The figures recorded by Innes (1935) of the blood picture of lambs affected with swayback are reproduced in the table: neutrophilia was apparent in some lambs and five out of the seven showed eosinopenia. Innes and Shearer (1940) presumed that the lower red counts were due to starvation. The possibility of worm infestation was not excluded.

Pregnancy Toxaemia. The haemogram of a ewe carrying twin lambs is recorded in the table; it agrees with the blood picture described by Clark (Groenwald, Graf, Bekker, Malan and Clark, 1941), who found that a relative and progressive neutrophilia, commencing several days before the onset of clinical symptoms, was the only change. The high red count arising in the ewe five hours before death presumably marks the onset of collapse.

Parturient Hypocalcaemia (*Lambing Sickness*). The only abnormality in the two cases recorded was a slight neutrophilia together with eosinopenia.

Double Scalp (*Scappie*). In addition to being affected with scappie, the two cases recorded unfortunately had high worm egg counts (2,000 and 11,000 per g.), and this forms an alternative explanation for the low red counts.

Inanition. Clark (Groenwald *et al.*), working on the blood picture of pregnancy toxaemia (Domsiekte), starved sheep that were known to be free from worms. He found that a relative neutrophilia occurred after about seven days' semi-starvation in pregnant ewes and after about 26 days in non-pregnant ewes. He made the important observation that after periods extending to 50 days on inadequate rations there was no alteration in the erythrocytic picture, as shown by haemoglobin estimation or haematocrit readings.

This observation supports those obtained in cobalt deficiency, scrapie, Solway pine, border pine and swayback, where inanition exists owing to the animal's inability to graze, and in which marked emaciation and debility

may exist in sheep that show no sign of anaemia. It also suggests that when anaemia is seen in some sheep in flocks affected with these conditions it may be due to an intercurrent infestation with worms.

Enzootic Toxaemic Jaundice. Chamberlin (1933). *Hypochromic Anaemia. Hyperchromic Anaemia of Pregnant Ewes.* Innes and Shearer (1940). These conditions are presumed to be due to metabolic disorders but have already been described under Anaemia (Holman, 1945).

DISEASES OF UNKNOWN AETIOLOGY

Photosensitisation (Yellowses). Two cases are recorded: in one, the only abnormality was eosinopenia; in the other, which was severely affected, there was a marked neutrophilic reaction and an abnormally long coagulation time. This abnormal coagulation time was confirmed by a second test carried out 20 minutes later, and was intelligible through the finding later at *post-mortem* examination of an enlarged " nutmeg " type of liver.

Enzootic Pneumonia. Cases from three outbreaks have been recorded and these have been arranged in the order that appears to portray the course of the reaction. In the first case examination was limited to a blood smear; in this there was a marked relative neutrophilia and a severe shift to the left but the total number of leucocytes appeared normal. In the second case a severe shift to the left was again apparent but the neutrophils were low in proportion and a leucopenia was recorded. In the third case haemograms from three sheep showed a leucopenia in which there was complete agranulocytosis. This sequence suggests that in this disease granular leucocytes are removed from the peripheral blood so fast that despite the increased production as shown by the severe shift to the left, the condition ends as an agranulocytosis.

DISEASE DUE TO POISON

Arsenic. Haemograms are recorded for two sheep given an intra-peritoneal injection of an arsenical compound. Unfortunately the preliminary red counts were already low. A slight neutrophilia is seen together with a steady fall in red count leading to regenerative changes.

Lead. Rupp (1928), quoted by Wirth, gave sheep 5 mg. of lead twice a week, sometimes by mouth and sometimes by subcutaneous injection. The red count was reduced, in one case to one-fifth of the original count, and between 18 to 32 days after the first dose punctate basophilia was observed, reaching a proportion up to eight corpuscles per 1,000; polychromasia and reticulocytes were present in the pre-experimental samples. Other authors failed to produce regenerative changes.

Phenothiazine. Two six-month-old lambs were each given a dose of 80 g. of phenothiazine and another lamb was given twice this amount. No alteration was observed in the blood picture.

Sulphanilamide. Three cases are recorded in the table. Little

change occurred in the leucocytic picture except for a terminal neutrophilia in the fatal case; there was no suggestion of agranulocytosis. Haemoglobinuria indicated haemolysis, but this was too slight to cause haemolytic anaemia.

SUMMARY OF SURVEY

Entero-toxaemic Diseases. In lamb dysentery where, presumably, *Cl. welchii* Type B toxin was predominant, a leucopenia without haemo-concentration occurred. The outbreak described by Hare and Glynn suggested that in some cases the *gamma* toxin might be predominant giving a reaction similar to Type C toxin. In pulpy kidney disease the reaction to Type D toxin was seen as a leucopenia together with haemo-concentration.

Pyogenic Organisms. There was a typical neutrophilic reaction varying in degree with the severity of infection and absent when infection was localised. Pyobacillosis involving lymph glands produced oligocythaemia.

Paratuberculosis. A variable neutrophilia occurred and there was a tendency towards hypochromic anaemia.

Fusiformis necrophorus Infection. A variable neutrophilia occurred with periods of leucocytosis. Oligocythaemia was progressive and terminated in an aplastic anaemia.

Viruses. No neutrophilic reaction, except that due to trauma, was observed. Eosinophils decreased during infection, except in scrapie, and increased during the recovery phase. Neutrophilia could be stimulated by intercurrent infections. In scrapie an eosinopenia and a slight neutrophilic reaction was usual for a period of a few days before death.

Rickettsia. With tick-borne fever, the bodies could be seen in neutrophils, monocytes and eosinophils during the febrile reaction, preceding the decrease in the proportion of neutrophil cells which usually amounted to neutropenia. Eosinophils decreased in numbers during the course of the disease and recovery was heralded by the usual increase.

Endo-parasites. Records from this survey and from a review of the literature show that neutrophilia may occur after some weeks in badly infested sheep. Eosinophils decrease in numbers—although in certain cases, with more mild infestations, this decrease is preceded by a transient eosinophilia: recovery is heralded by the usual eosinophilia. Where *H. contortus* is predominant anaemia is post-haemorrhagic and may be macrocytic; with *liver flukes* anaemia may be aplastic, and with other species there is a tendency to a hypochromic anaemia.

Metabolic Disorders. Excluding enzootic toxaemic jaundice, *hyperchromic anaemia* and *hypochromic anaemia*, no changes in the cytology were observed except those attributed either to starvation or worm infestation.

Unknown Aetiology. Yellowses, where severe, showed a marked

neutrophilia, eosinopenia, and prolonged coagulation time. Enzootic pneumonia was accompanied by a marked neutrophilic shift, despite which leucopenia, and finally agranulocytosis, was observed.

Poisons. No general reaction can be laid down.

GENERAL DISCUSSION

Haematology is based on measurement, and until the measurements for each constituent in each species are compared to absolute standards, rather than relative ones, veterinary literature will continue to contain different articles with the same figure recorded by one worker as, for example, a leucopenia, and by another as a normal leucocytic count. It is evident that comparative measurements between flock and flock, or herd and herd, ignore all environmental factors and allow full play for personal bias.

In these studies, therefore, an attempt has been made to set absolute standards for the sheep, acting on the principle that such standards can be adjusted as knowledge in the diagnosis of minor ailments is improved, but that in the meantime they afford a practical means of detecting gross changes and subordinating personal bias.

That the standards set are to some extent incorrect is suggested by the frequency distribution given for apparently healthy animals (Holman, 1944a) where, for example, the histogram for neutrophils shows a negative skew and the haemoglobin a positive one. The possible explanation of this bias towards neutrophilia and towards anaemia is suggested in this survey which shows that worm infestation and certain types of deficiency in diet bias the figures in that direction. Hence it would appear likely that if sheep suffering from deficient diet and worm infestation could be eliminated from among apparently healthy sheep, the normal range for some, perhaps most, constituents could be reduced.

To gain some perspective on the alteration in blood picture due to disease it is advantageous to compare these haemograms with those recorded for man, the only species for which extensive data are available. The difference in reaction in the case of pyogenic organisms and the difference in corpuscular shape in macrocytic anaemia between sheep and man has already been described (Holman, 1944c, 1945).

With regard to virus diseases, these, in man, generally produce a neutropenia, except where the central nervous system is involved, when a neutrophilia is observed. In sheep, although there is no neutrophilia, neutropenia is not apparent, nor is there a leucocytosis in virus infections that affect the central nervous system, such as louping ill and scrapie. In intestinal parasitism, *H. contortus* has its analogue in hookworm infestation in man, but again there is a difference, for in man the anaemia is hypochromic and there is a high eosinophil count, while in sheep there is a tendency for the anaemia to become hyperchromic and there is an eosinopenia. A

hyperchromic anaemia does, however, occur in man due to the depredations of *Diphyllobothrium latum*, but here the anaemia is thought to be due to the failure of the gut to assimilate the haemopoietic principle (Whitby and Britton, 1939), whereas in *H. contortus* infestations in sheep the macrocytic anaemia has been regarded as an attribute of rapid haemopoiesis. The hyperchromic anaemia of pregnant ewes has an analogy in a nutritional macrocytic anaemia occurring in pregnant women and further it is of interest to note that a nutritional macrocytic anaemia also occurs in the dog where it is produced by the absence of nicotinic acid (Hadfield and Garrod, 1943). Agranulocytosis in man occurs either as an idiopathic disease associated with necrotic ulcerations or as a result of poisoning by various substances that include sulphanilamide. In the sheep, the evidence is against the hypothesis that even fatal doses of sulphanilamide produce agranulocytosis, but the condition is seen in both enzootic pneumonia and in fatal toxaemia due to *Cl. welchii*, Type A, the latter being an organism that produces intense leucocytosis in man. These selected comparisons make it clear that, although analogies often exist between the blood pictures of man and sheep, as in all other experimental work it is a dangerous expedient to argue from one to the other on the assumption that the blood picture is similar in the two species.

Finally, the results indicate that in experimental work on sheep diseases, the haemogram—limited, where appropriate, to total and differential leucocyte counts and an estimation of haemoglobin—can be used as a guide to the course and an indication of the severity of the disease, for in work of this nature the use of the wide normal range can be avoided as a criterion, and small differences denoting an interference with the normal biological variation can be detected by using a table giving the Maximum Admissible Deviations (Holman, 1944b).

SUMMARY

1. A survey is presented of blood pictures occurring in the diseases of sheep.

2. Blood pictures in certain cases have been compared with those in man and it is concluded that it would be fallacious to argue from the one species to the other until a similarity in reaction had been verified.

3. The survey affords evidence in support of Clark's (Groenwald *et al.*) contention that semi-starvation does not cause anaemia in sheep.

4. It is contended that the use of the M.A.D. as a criterion renders the haemogram of value as a sensitive indication of both the course and severity of disease in sheep used for experiment.

TABLE

SELECTED HAEMOGRAMS FOR SHEEP DISEASES

Disease	*No. of Sheep Sampled*	*Red Count*	*White Count*	*Platelet Count*	*Metamyelocytes*	*Polymorphonuclears*	*Lymphocytes*	*Monocytes*	*Eosinophils*	*Basophils*	*Coagulation Time*	*Fragility*	*Specific Gravity*	*Haemoglobin g. per 100 ml.*	*Packed Cell Volume*	*Viscosity*	*Remarks*
BACTERIAL DISEASES																	
Enterotoxaemia. (Cl. welchii)																	
Type A toxin	1	2·1	6·2	430	0	3	95	2	0	0	8·5	0·75	1037	4·6	4	—	3 hours after inoculation.
Fatal doses, intravenously...	1	8·6	8·9	200	0	2	98	0	0	0	2·5	0·55	1042	8·1	21	—	1 hour after inoculation.
	—	5·2	8·4	300	0	8	91	1	0	0	2·5	0·55	1040	7·0	15	—	2 hours after inoculation.
Type B toxin	3	13·5	11·0	360	—	33	63	1	3	0	4·3	0·58	1055	—	32	—	Pre-experimental.
Fatal doses, intravenously...	—	12·1	2·8	740	—	9	89	1	1	0	5·5	0·60	1050	—	27	—	Hours after inoculation.
Type C toxin	4	11·9	11·0	310	0	30	56	3	10	0	4·1	0·52	1054	11·9	30	—	Pre-experimental.
Fatal doses, intravenously...	—	12·7	13·8	200	0	52	46	2	1	0	2·3	0·55	1053	12·3	32	—	Hours after inoculation.
Type D toxin	5	10·8	10·7	(223)	0	32	57	4	6	1·2	(3·5)	(0·54)	(1052)	10·9	26	(5·1)	Pre-experimental.
Fatal doses, intravenously...	—	13·2	6·9	(145)	0·5	35	62	2·2	0·2	0·2	(2·6)	(0·52)	(1062)	13·2	33	(6·3)	Hours after inoculation.
Lamb Dysentery	5	9·7	5·8	—	—	30·5	59·8	8·4	0·2	1	—	—	—	—	—	—	Early infections.
Hare and Glynn, 1927	7	9·5	5·9	—	—	28	63·9	6·5	0·9	0·8	—	—	—	—	—	—	Healthy controls, same flock.
	9	8·8	11·5	—	—	54·7	37·2	7·2	0·4	0·4	—	—	—	—	—	—	Few hours before death.
	3	10·1	12·3	—	—	40·2	51·5	6	1·5	0·7	—	—	—	—	—	—	Healthy controls, same flock.
Fraser, 1931 ...	10	(10·6)	(3·4)	—	—	37·3	54·6	6·5	1·6	0·1	—	—	—	—	—	—	Affected lambs.
	4	—	—	—	—	16·1	75·8	6·9	0·8	0·5	—	—	—	—	—	—	Acutely affected.
Pulpy Kidney Disease ...	1	14·0	2·2	—	4	11	77	8	0	0	—	0·50	—	—	41	8·5	Appeared healthy 3½ hours previously.
Staphylococcal Infections																	
Gangrenous Mastitis	1	12·0	19·4	—	1	48	48	1	2	0	—	0·40	—	13·8	32	10	—
	1	9·4	3·4	—	11	45	41	3	0	0	—	0·45	—	9·8	26	6·2	Toxic changes in neutrophils.
	1	10·4	16·5	—	1	70	27	0	1	0	—	0·40	—	12·6	27	6·5	—
	1	10·0	14·0	—	3	47	45	2	2	1	—	0·45	—	11·0	25	6·0	—
Tick Pyaemia	1	14·0	21·5	—	4	46	43	7	0	0	—	—	—	—	31	—	Toxic changes in neutrophils.
	1	11·8	15·6	170	2	23	70	5	0	0	2·5	0·40	—	13·2	24	7·1	T.B.F. bodies.
Joint Ill	1	6·5	9·8	—	4	40	51	1	3	1	—	0·50	1045	—	10	3·7	PB++ P+.
	1	—	—	—	0	16	83	1	0	0	—	—	—	—	—	—	T.B.F. bodies.
	1	—	—	—	0	23	77	0	0	0	—	—	—	—	—	—	T.B.F. bodies.
	1	—	8·0	—	—	45	48	6	0	1	—	—	—	—	—	—	—

Paratuberculosis (*Johne's Disease*)	1	11·0	7·5	60	3	54	37	6	0	0	2·0	0·50	1047	8·7	29	4·7	—
	1	6·7	12·2	610	48	26	22	3	0	1	3·0	0·55	1037	6·3	17	3·4	—
	1	9·8	20·4	80	9	86	3	2	0	0	1·5	0·50	1042	8·3	26	3·0	—
	1	7·1	7·3	220	19	12	47	22	0	0	2·0	0·50	1037	6·7	18	2·8	—
	1	11·0	11·6	—	5	70	22	2	1	0	—	0·65	1040	9·3	25	5·0	—
	1	8·8	6·0	—	0	54	38	4	4	0	—	0·65	1045	8·2	25	3·2	—
	1	10·5	8·6	—	10	40	40	8	2	0	—	0·65	1047	9·9	27	4·8	—
	1	7·5	22·0	—	2	59	33	6	0	0	3·0	0·40	—	—	18	3·7	—
	1	5·5	17·1	—	1	53	43	3	0	0	3·0	0·45	—	7·0	18	2·6	—
	1	8·3	9·2	—	1	57	24	0·5	0·5	0	—	—	—	8·6	24	3·2	—
	aver.	8·6	12·1	242	18	51	31	5·6	0·7	0	2·5	0·55	1043	8·0	23	3·7	—
Pyobacillosis (*A. lignieresi*)	3	11·8	12·3	—	1	29	53	3	13	1	—	—	—	11·8	37	—	Healthy, same flock.
	3	8·7	13·5	—	4	37	44	7	7	2	—	—	—	8·2	25	—	Glands affected.
	1	11·4	6·1	—	0	41	53	6	0	0	2·0	0·50	—	11·0	18	5·0	Abscess in jaw only.
	1	6·2	8·3	—	0·5	37	58	4	0·5	0	1·5	0·55	—	14·2	20	4·4	Abscess in jaw only.
Fusiformis necrophorus infection in lambs	1	10·8	10·6	—	4	32	54	3·5	3·5	3	—	0·55	1055	11·4	25	4·1	40 days before killed.
	—	6·1	10·5	—	0	22	60	4	12	2	—	0·55	1057	9·2	22	4·4	33 days before killed.
	—	5·4	22·5	—	4	61	31	3	1	0	—	0·55	1047	6·5	17	4·7	26 days before killed.
	—	6·3	7·7	—	1	33	61	4	1	0	—	0·55	1045	6·2	17	4·4	5 days before killed.
	1	7·4	11·1	—	0	37	59	2	2	0	—	0·55	1055	9·2	21	3·3	23 days before death.
	—	4·7	16·9	—	3	67	25	5	0	0	—	0·55	1050	5·0	16	3·2	19 days before death.
	—	3·7	14·7	—	26	57	13	3	0	1	—	0·50	1052	5·4	16	4·7	11 days before death.
	—	3·9	10·2	—	10	56	33	1	0	0	—	0·55	1037	4·8	12	3·4	1 day before death.
	1	13·0	31·8	—	16	45	34	6	0	0	—	0·50	—	16·0	38	13·5	Lamb unable to stand. T.B.F. bodies present.
VIRUSES AND RICKETTSIA																	
Louping Ill. (*Trembling*) ...	3	—	10·1	—	3	24	60	5	1	1	—	—	—	12·7	—	—	Pre-inoculatioo.
	—	—	11·2	—	4	29	61	5	1	0	—	—	—	12·9	—	—	2 days after. Reaction to inoculation.
	—	—	10·5	—	1	37	57	4	1	0	—	—	—	12·2	—	—	10 days after inoculation.
	—	—	10·2	—	0	31	62	2	5	0	—	—	—	11·4	—	—	20 days after inoculation, recovery stage.
Contagious Pustular Dermatitis	4	9·6	4·0	—	0	17·5	71	3	8	0·5	2·6	0·60	1045	10·2	24	7·0	Pre-inoculation.
	—	9·2	6·6	—	3·1	37	53·5	1·7	6	0·2	2·2	0·60	1045	10·5	24·5	7·1	2 days after inoculation.
	—	9·6	9·2	—	1·9	29·5	60·6	3	2·9	0·1	2·3	0·60	1046	10·6	23	6·5	4 days after inoculation.
	—	10·6	8·1	—	2·4	29·1	64	1	3	0	2·5	0·60	1044	11·0	26·5	6·9	8 days after inoculation.
	—	9·9	5·7	—	1·5	26·5	66	1·5	4·2	0·4	2·5	0·60	1043	10·6	25	6·6	10 days after inoculation.
	—	9·7	8·0	—	0·4	20·1	68·4	2·1	9	0	2·7	0·60	1042	9·8	24	6·4	14 days after inoculation.
Scrapie	15	11·9	8·8	—	1·2	31·7	60	2·5	4·3	0·4	2·3	0·51	—	12·1	26	6·9	When first observed ill.
	10	11·7	8·2	—	1·1	31·2	59·5	2·6	4·6	0·3	2·75	0·50	—	11·4	29	6·0	Last samples except those below.
	5	11·4	10·0	—	2·6	46·6	47·4	3·4	0	0	2·7	0·52	—	12·0	29	7·9	Within 10 days of death.

TABLE—*continued*

SELECTED HAEMOGRAMS FOR SHEEP DISEASES

Disease	*No. of Sheep Sampled*	*Red Count*	*White Count*	*Platelet Count*	*Metamyelocytes*	*Polymorphonuclears*	*Lymphocytes*	*Monocytes*	*Eosinophils*	*Basophils*	*Coagulation Time*	*Fragility*	*Specific Gravity*	*Haemoglobin g. per 100 ml.*	*Packed Cell Volume*	*Viscosity*	*Remarks*
Tick-borne Fever	1	—	9·2	—	0	2	84	14	0	0	—	—	—	9·6	—	—	At time of most pronounced neutropenia.
	1	—	8·4	—	0	18	77	4	1	0	—	—	—	11·9	—	—	At time of most pronounced neutropenia.
	1	—	8·4	—	0	6	91	3	0	0	—	—	—	11·7	—	—	At time of most pronounced neutropenia.
	1	12·0	5·8	110	1	4	87	7	0	0	4·0	0·55	1060	—	33	6·2	At time of most pronounced neutropenia.
HELMINTHIASIS AND COCCIDIOSIS																	
1. *Haemonchus contortus predominant*																	
Flock A. Macrocytic Anaemia	3	6·4	6·9	—	0	30	56	6	6	2	—	0·53	—	9·7	27	7·2	M.C.V. 42 c.μ.
Hypochromic Anaemia	3	8·7	8·8	—	0·3	34	57	5	2	1	—	0·52	—	9·2	30	7·1	M.C.H.C. 30 per cent.
Neutrophilia ...	1	9·2	8·8	—	3	48	45	3	0	1	—	0·50	—	10·9	25	7·6	—
Routine Diagnosis.																	
Macrocytic Anaemia ...	1	2·3	4·8	—	5	68	24	2	0	1	1·5	0·50	—	4·2	10	3·6	M.C.V. 44 c.μ.
Hypochromic Anaemia ...	2	8·5	8·2	—	3	46	41	6	3	0	(1·5)	(0·45)	—	7·2	22	(3·5)	M.C.H.C. 33 per cent.
Anhydraemia	1	15·0	6·2	—	0	54	42	4	0	0	3·0	0·50	—	12·2	30	5·0	—
2. *Other species predominant*																	
Chabertia ovinus	1	6·8	8·8	—	20	26	54	0	0	0	3·5	0·50	1040	6·7	21	2·4	—
Ostertagia circumcincta and Trichuris ovis	1	—	15·8	—	11	49	29	11	0	0	—	—	—	—	—	—	P + PB +
Ostertagia circumcincta and Trichostrongyles	1	8·5	6·2	—	8	58	30	2	0	2	—	—	—	10·8	25	—	—
Trichostrongylus axei ...	1	11·0	12·1	—	13	42	42	3	0	0	3·0	0·50	—	—	30	6·2	—
Lung worms and tapeworms	1	10·1	14·8	—	3	42	54	1	0	0	—	—	—	8·9	26	—	—
Lung worms and stomach worms	1	9·5	8·6	—	3	51	40	6	0	0	—	—	—	8·3	20	—	—
Lung worms, liver flukes and intestinal worms	1	14·5	10·5	250	1	46	48	4	1	0	2·5	0·55	1060	13·8	35	7·2	Anhydraemia.
	—	4·1	9·0	—	4	50	43	3	0	0	2·0	0·55	1032	5·2	14	3·6	1 month later aplastic anaemia.
Coccidiosis	1	7·0	6·3	—	1	34	55	5	3	2	2·5	0·45	—	10·6	25	3·7	Anaemia.
	1	13·8	12·4	—	0	48	40	4	7	1	1·5	0·50	—	13·4	32	5·3	Anhydraemia.

Diseases associated with Metabolic Disorders																	
Cobalt Pine	10	11·6	—	—	—	37	53	7	2	1	1·8	0·50	1053	10·6	30	5·2	Very few band forms.
Solway Pine	22	10·3	7·1	—	0·6	38	58	2	1·5	0·2	2·7	0·55	1046	9·2	22	5·0	—
Swayback. (Innes, 1935) ...	7	9·7	8·0	—	—	41	53·5	4	1	0·5	—	—	—	—	—	—	PB. + Anisocytosis.
Pregnancy Toxaemia... ...	1	11·5	9·2	—	7	68	20	5	0	0	2·5	0·50	1062	15·0	37	23·0	22 hours before death.
	—	13·1	13·2	—	40	42	10	8	0	0	2·5	0·55	1070	17·1	43	20·0	5 hours before death.
Parturient hypocalcaemia	1	—	—	—	4	59	36	1	0	0	—	—	—	9·9	—	—	Unable to stand.
(Lambing sickness)	1	—	—	—	2	45	51	0	0	2	—	—	—	10·7	—	—	Unable to stand.
Double Scalp	1	8·3	6·9	—	0	24	72	3·5	0·5	0	2·5	—	1050	9·5	24	6·3	—
(Scappie)	1	4·4	7·8	—	1	39	53	7	0	0	3·0	—	1035	4·2	12	4·1	Also struck by blow-fly.
Diseases of Unknown Aetiology																	
Photosensitisation: *Yellowses*	1	9·8	10·3	100	—	46	54	0	0	0	2·0	0·45	1042	9·1	23	3·7	—
	1	12·5	23·8	300	7·5	65	19	2	6	0·5	7·0	0·55	1057	13·6	33	6·6	—
Enzootic Pneumonia	1	—	—	—	66	20	12	2	0	0	—	—	—	—	—	—	No leucopenia apparent.
(All cases fatal)	1	11·2	3·2	D	34	9	46	11	0	0	3·0	0·50	1052	12·8	30	5·0	Toxic changes in neutrophils.
	3	9·4	2·4	—	0	0	87	13	0	0	3·0	0·50	—	8·9	32	—	—
Diseases caused by Poisons																	
Arsenical Poisoning	1	7·9	11·0	—	2	14	76	2	5	1	—	—	—	7·6	—	—	Pre-experimental.
	—	5·0	15·5	—	1	51	45	2	1	0	—	—	—	5·8	—	—	Day after administration.
	—	5·4	9·0	—	1	38	59	1	1	0	—	—	—	5·4	—	—	2 days after.
	—	5·4	8·5	—	2	33	54	5	5	1	—	—	—	4·7	—	—	3 days after. PB (+).
	—	4·7	8·5	—	0	24	71	1	3	1	—	—	—	5·2	—	—	7 days after. PB +.
	—	5·5	8·5	—	0	21	77	0	1	1	—	—	—	6·4	—	—	12 days after. PB (+).
	—	6·8	12·0	—	0	26	69	3	1	1	—	—	—	6·8	—	—	15 days after. PB —.
	1	7·4	10·0	—	0	17	75	3	1	4	—	—	—	8·8	—	—	Pre-experimental.
	—	6·3	16·5	—	10	62	26	2	0	0	—	—	—	6·3	—	—	1 day after.
	—	5·7	8·3	—	6	24	54	4	2	0	—	—	—	5·7	—	—	2 days after. PB (+) P (+).
	—	4·8	8·0	—	2	29	66	2	1	0	—	—	—	5·8	—	—	7 days after. PB (+).
	—	6·2	6·0	—	3	22	64	3	5	3	—	—	—	6·5	—	—	15 days after. PB —.
Sulphanilamide Poisoning	1	11·4	6·9	650	2	47	50	1	0	0	2·0	0·55	1057	11·0	28	3·8	Haemoglobinuria.
	1	8·4	7·7	50	6	34	55	2	2	1	1·5	0·60	1052	10·8	26	5·2	Haemoglobinuria.
	1	7·4	14·2	50	4	42	48	5	1	0	3·0	0·55	1042	7·6	20	4·1	2 days before death.
	—	6·6	14·5	800	1	42	51	5	0	1	3·5	0·55	1042	7·0	17	3·7	1 day before death.
	—	7·1	14·2	550	2	53	37	6	0	2	3·5	0·55	1045	8·6	21	3·9	Day of death.
Approximate Standards																	
Normal Means	—	11·5	9·2	—	0·5	24	67	2	4	0·5	2·5	0·40	1052	12·4	30	5·8	M.C.V. 27 M.C.H.C. 41
Daily Variation. M.A.D. (95 per cent.)	—	2·8	4·0	—	1·0	16	16	3·3	8	1·6	1·5	0·03	1008	1·9	7	2·4	M.C.V. 7·7 M.C.H.C. 9·7.
Abnormally High	—	15·0	13·0	—	6	47	85	14	8	4	6·5	0·70	1067	16·5	39	9·0	M.C.V. 38.
Abnormally Low	—	8·0	4·0	—	—	10	—	—	—	—	0·5	0·35	1040	9·0	23	4·0	M.C.H.C. 33.

D = Diversity of shape and size. ( ) = Figure not based on full number of sheep.

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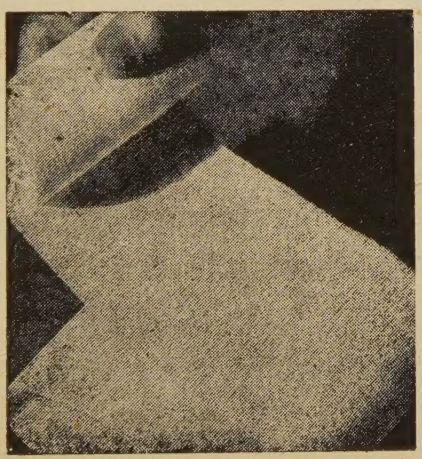

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